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*(Recognosce notum, ignotum inspice)*

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STUDIES ON *SCLEROTINIA* AND *BOTRYTIS*II. DE BARY'S DESCRIPTION AND SPECIMENS OF  
*PEZIZA FUECKELIANA*

By P. H. GREGORY

*Agricultural Research Council Research Officer at Rothamsted  
Experimental Station, Harpenden, Herts*

(With Plates I-III and 1 Text-figure)

*Botrytis cinerea* Fr. is a facultative parasite with a wide host range in temperate regions. The perfect stage was named *Peziza fuckeliana* by de Bary in 1866, and transferred to the new genus *Sclerotinia* by Fuckel in 1869. The connexion between *Botrytis cinerea* and *Sclerotinia fuckeliana* was generally accepted until the beginning of this century, when the adoption of pure-culture methods in plant pathology aroused doubts of the correctness of the conclusions de Bary drew from his observations. Isolates of *Botrytis cinerea* could be maintained *in vitro* for many years without producing apothecia from the sclerotia. Authorities such as Lind (1913) and Brierley (1931) even denied the existence of a *Sclerotinia* stage, Klebahn (1930) regarded the question as needing a new investigation, and the theory fell into disrepute.

However, apothecia were produced in pure cultures of *Botrytis* by several workers, including Seaver and Horne (1918), Seaver (1947), Godfrey (1919, 1923) and van Beyma Thoe Kingma (1927). The connexion was firmly established when Drayton (1937) with *B. convoluta*, and Groves and Drayton (1939) with *B. cinerea*, studied the conditions under which apothecia were formed in culture, and described how they could be produced at will. Their method involved spermatizing sclerotia of *Botrytis* isolates by microconidia from other compatible isolates, combined with suitable cold treatment. The failure of earlier workers to obtain the *Sclerotinia* stage from pure cultures of *Botrytis* is thus due directly to the purity of the technique used, which provided no mechanism for the transfer of microconidia to the receptive bodies of the same or other isolates. The methods of Drayton and Groves were subsequently used by Buddin (in Dennis & Wakefield, 1946) with a new species *Sclerotinia draytoni* from *Gladiolus*. The connexion has also been confirmed by me for *Sclerotinia narcissicola*, *S. polyblastis*, *S. sphaerosperma*, and an unnamed species of *Sclerotinia*, with apothecia produced out of doors from naturally infected plant material (Gregory, 1940, 1941).

The question arises whether all species of *Sclerotinia* arising from sclerotia of *Botrytis cinerea* belong to *Sclerotinia fuckeliana*. The problem has been complicated by uncertainty as to what fungus de Bary had, because his specimens and description have been overlooked hitherto. Saccardo cites 'De Bary, *Morph. Pilz.* p. 30' (1866) for the description, but in the passage

referred to de Bary merely stated that '*P(eziza) Fuckeliana* n.sp.' was to be described in detail elsewhere. Search by me in the usual sources failed to reveal any recognizable description by de Bary of *Sclerotinia fuckeliana*. Eventually Dr G. R. Bisby noticed a figure in Zopf's *Die Pilze*, accompanied by a reference to an obscure paper of de Bary's which proved to contain the missing description and illustration of *Peziza fuckeliana*. Dr J. Ramsbottom then drew my attention to de Bary's collection of microscope slides in the Department of Botany, British Museum (Natural History). This contains a slide with sections of apothecia labelled '*Peziza fuckeliana*, 22/10/64', which must constitute the type specimen. The following account summarizes the information on this fungus available from various sources, and should help to clarify the position of apothecia now obtained in culture.

1797. C. H. Persoon. *Tentamen dispositionis methodicae fungorum*. Lipsiae.

On p. 40 the fungus is referred to as '*Botrytis. B. cinerascens, sparsum, ramosum ex albido cinerascens. tab. 3. fig. 9. (I in fol. putrid. brss. olerac.)*', but p. 47, fig. 9, refers to this fungus as '*Botrytis cinerea...*' and this is apparently the first use of the name.

1801. C. H. Persoon. *Synopsis methodica fungorum*.

P. 690 gives the following description:

'*Botry. cinerea: congesta late effusa ramosa cinerea*. Disp. fung. p. 46, t. 3. fg.

'Hab. in *Cucurbitus* putrescentibus, et ad caules *Brassicae oleraceae*. Sub lente illustrata formam distinctam sistit, ceteroquin *Byssos* similis.'

1832. E. Fries. *Systema Mycologicum*, III, ii.

On pp. 396-7 under *Botrytis* the fungus is described as follows:

'*B. cinerea*, floccis fertilibus gregariis sub simplicibus cinereis, mox strangulatis, sporidiis hinc inde accumulatis globosis albidioribus.

'*B. cinerea*. Pers. Syn. p. 690 disp. meth. t. 3. f. 10. [? fig. 9] haud bona! Alb. et Schwein. Consp. p. 363. Schum. Saell. 2. p. 238. Link. Spec. 1. p. 63.

'A praecedente [*B. grisea*] longe removeri non debet. Flocci steriles in hac saepe etiam evanescent, sed fertiles in densam silvulam, lineam altam, stipantur, jam nudo oculo distinguuntur farinosi; omnino simplices aut uno alterove ramo simplici aucti. Sub. micr. comp. flocci adulti semper strangulati, articulis alternis constrictis, alternis compressis, ut quasi *Alternaria* sui generis. Sporidia glomerata, globosa, alba, per aetatem cinerea. Duae praecipue sunt hujus speciei formae habitu valde recedentes, sed characteribus haud separandae; altera ad caules exsiccatos nudo oculo magis fructiculosa, rigida, cinerea; altera ad caules putredine fere dissolutos, haec mollis, mucidinea, fuligineo-cinerea. Ad caules plantarum emortuos vulgatissima omni anni tempore (v.v.).'

This description is the nomenclatural type of *Botrytis cinerea* under the Rules of Botanical Nomenclature, until a type or lectotype specimen may be found or designated. It is of interest to note that even as early as the time of Fries it was recognized as being a variable species.

#### *Sclerotinia fuckeliana*

1866. A. de Bary in W. Hoffmeister's *Handbuch der Physiol. Botanik*, 2. Bd.

1866. *Morphologie und Physiologie der Pilze, Flechten und Myxomyceten*, Leipzig, 8. xii u. 316 S.

The first reference to the fungus appears on p. 30 in a list of fungi which arise from sclerotia: '*P. Fuckeliana* n.sp.<sup>1</sup> (*Scl. echinatum* Fuck.)'. The footnote reads:

<sup>1</sup> Mit obigem Namen bezeichne ich die anderweitig ausführlich zu beschreibende der *P. Candolleana* nahe verwandte *Peziza*, die auf Rebenblättern kleine schwarze Sclerotien (*Scl. echinat.* F) bildet.'

Elsewhere in this volume there are various references to *Peziza fuckeliana*, as well as two illustrations, none of which, however, can be considered as providing a recognizable description. Fig. 12 on p. 31 shows a thin section through a sclerotium magnified 390 times, and Fig. 17 on p. 39 shows a section through a sclerotium and young apothecium magnified 20 times, both of which are reproduced in the English translation (1887) of the enlarged later edition (1884) of this work. This first reference to *P. fuckeliana* by de Bary was cited as the original description of the species by Saccardo (1889) and by Rehm (in Rabenhorst 1896), but as de Bary merely stated that the fungus was to be described in detail elsewhere this entry is obviously a *nomen nudum*.

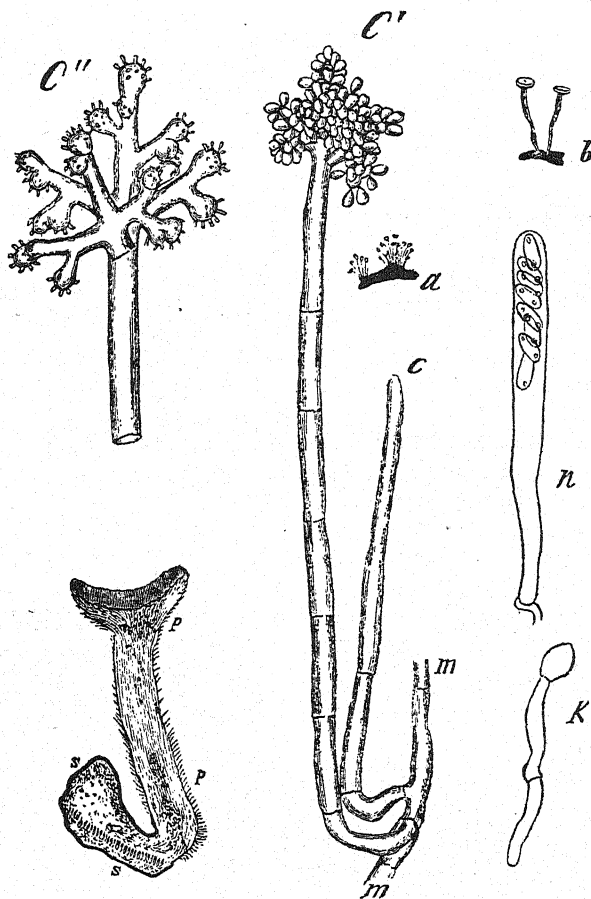
The first account of the biology of *P. fuckeliana* begins on p. 201. De Bary states that the sclerotia of this fungus are formed in tissues of dead vine leaves in autumn and winter. If they are placed on the surface of moist soil, either in a ripe fresh condition, or after several months' dry storage, they produce conidial fructifications in the course of twenty-four hours with all the characteristics of *Botrytis cinerea* Pers. If, however, the sclerotia are buried a few millimetres or a centimetre under the surface of the soil, stalked plate-like ascus-bearing cups are formed in summer instead of conidiophores. Numerous cultures from germinating ascospores gave only sclerotia without the formation of conidia. The question if the *Botrytis* conidiophores, known to grow out from sclerotial forms like *Sclerotium durum* and *S. bullatum* also belong to the *Formenkreis* *Peziza fuckeliana* was still to be decided.

1869. A. de Bary. Ueber Schimmel und Hefe. in Virchow und Holtzendorf's Samml. gemeinverst. wiss. Vortr. Serie IV, Heft 87 and 88, pp. 551-630. Berlin (Separate pagination 1-78).

This semi-popular account of fungi and yeasts with special reference to fermentation processes was made the occasion for fairly full descriptions of the biology of some typical moulds and yeasts: *Aspergillus glaucus*, *Botrytis cinerea*, *Mucor stolonifera*, *M. mucedo*, *Penicillium glaucum*, *Oidium lactis* and *Saccaromyces cerevisiae*. Nine pages are devoted by de Bary to *Botrytis cinerea* and *Peziza fuckeliana* and with his 'Fig. 2 *Botrytis cinerea*', reproduced here as Text-fig. 1, presumably forms the description of *Peziza fuckeliana* n.sp. to which a passing reference was made in 1866. The description is diffuse and its main points relevant to *Sclerotinia fuckeliana* are as follows.

*Botrytis cinerea* is widespread on many different substrata but de Bary definitely states that his description is confined to the one which is almost always to be found on dead, damp, fallen vine leaves. The development of conidiophores, their evanescent terminal bladders on which the conidia are formed and the formation of sclerotia in late autumn after the fall of

the leaves are described. On the ground, if warm weather intervenes before the sclerotia are ripe, numerous clusters of *Botrytis conidiophores* may develop on the sclerotia (Text-fig. 1a) and the sclerotium subsequently shrivels. But if, after months of ripening, the sclerotia are placed on damp soil in summer or autumn a bundle of hyphae pushes through the rind of



Figur 2. *Botrytis cinerea*.

a u. b. Natürliche Größe; Sclerotien, aus denen bei a Conidienträger, bei b Schlauchfrüchte hervordringen. c, c' Conidienträger (c' mit eben reifen Conidien) von dem Myceliumfaden m entspringend (Vergr. etwa 200). C'' Ende eines Conidienträgers mit dem ersten Beginn der Conidienabschnürung auf den Zweigenden. k Keimende Conidie (Vergr. 300). — d (schwach vergr.) Durchschnitt durch ein Sclerotium s, aus welchem ein sehr kleiner Schlauchträger (p, p) hervordringt. n (Vergr. 390) Einzelnr Sporenschlauch mit 8 reifen Sporen.

Text-fig. 1. *Botrytis cinerea*.

the sclerotium, elongates and spreads out at the free end as a flat plate-like disk on the free surface of which are produced paraphyses and asci (n) containing eight oval spores. When ripe the free end of the ascus bursts and the spores are scattered to a great distance. Small sclerotia produce only one ascophore, larger sclerotia two to four (b). The ascophore, when full

grown, has a stalk one or more millimetres long and the diameter of the disk is one-third to three millimetres (seldom more). It is stated as a matter of special interest that in suitable media, such as on the damp injured surfaces of vine leaves, both conidia and ascospores put out germ tubes and grow directly into mycelial hyphae which can form conidiophores and finally sclerotia.

The mycelium and conidiophores on vine leaves are indistinguishable from the universally distributed mould on dead parts of plants of every kind. The sclerotia are also similar in form, but may be somewhat larger on other substrata, and produce masses of conidiophores like the form described on vine leaves. The stalked ascophores on the contrary have seldom been observed on other substrata, and when found are always very similar to those which originate in vine leaves, though differing from them in many details. 'It is possible that these various ascus fruit bodies belong to different but closely related species, although their sclerotial and conidial forms must all be recognized as *Botrytis cinerea*. This is why the description has been confined to the form on the vine whose development is well-known. Sclerotia are formed only on solid vegetable tissues such as leaves, pumpkins and thick stalks, while on delicate parts like flowers only mycelia and conidiophores are formed. The conidiophores, sclerotia and ascus-fruits were formerly regarded as distinct fungus species belonging to different genera, *Botrytis* (*B. cinerea*, *B. vulgaris*, etc.), *Sclerotium* (*S. durum*, *S. echinatum*). The former generic name is now applied to distinguish an organ or developmental stage which is present in many species; while the ascus-fruit belongs to the genus *Peziza*. It is the special form on vine leaves, which is named *Peziza Fuckeliana*.'

Thus, although not mentioned in the legend to 'Figur 2', the apothecium and ascus with ascospores are definitely stated in the text to be *P. fuckeliana* on vine leaves, the connexion being proved by observing the development of conidiophores on mycelia derived from ascospores.

Little more need be added to the description than to draw attention to the hairs on the stalk depicted below the apothecium and to give the measurements of the ascospores, which according to magnification indicated in the legend are about  $12 \times 5 \mu$  while in the ascus. The single ascus is  $130 \mu$  long. The drawing is presumably made from the type specimen which is mounted in glycerine.

1869. *L. Fuckel. Symbolae Mycologicae. Beiträge zur Kenntnis der rheinischen Pilze. Wiesbaden. Jahrb. d. Nassauischen Ver. f. Naturk. xxiii u. xxix, p. 330.*

Fuckel erected the new genus *Sclerotinia*, the first-mentioned species of which (and generally taken as the type of the genus) is *S. Candolleana* (Lév.) Fuckel, and the second:

'*S. Fuckeliana* (de By.).—*Peziza Fuckeliana* de Bary. *Morph. u. Physiol.* p. 30. (Fung. integr.)—I. Mycelium quiescens, *Sclerotium echinatum* Fuckel, *E.F.N.* Nr. 215.—F. rh. 1478—An faulenden Blättern von *Vitis vinifera*, selten, im Frühling. Um Oesterreich. II. Fungus conidiophorus (?). *Botrytis cinerea* Pers. Syn. p. 690. *Polyactis* Autor. pl.—F. rh. 148.—Auf dem *Scl. ech.*, sehr häufig.

'Die Schlauchform sah ich noch nicht.'



On p. 458 Fuckel adds a note to p. 330: 'Nach de Bary's neuesten Untersuchungen ist die genetische Beziehung dieser hier zusammengestellten Entwicklungsstadien ausser allen Zweifel gestellt.'

1872. *A. de Bary*. On Mildew and Fermentation. *Quarterly German Magazine* (Berlin), II, 153-226 (separate pagination 1-76).

This is a literal English translation of de Bary's 'Ueber Schimmel und Hefe' 1869, and contains 'Figur 2. *Botrytis cinerea*', apparently reprinted from the original wood block. Many technical terms are incorrectly translated.

1875-6. *M. C. Cooke*. Carpology of *Peziza*. *Grevillea*, IV, 132.

The legend to Pl. LXV, fig. 281, reads: '*Peziza Fuckeliana*. D'By. fide Dr. Bary.'

The figure of a single ascus is apparently copied from de Bary's 1869 paper, but has in addition two ascospores lying outside the ascus. The ascus measures  $140\mu$ , according to the scale given by Cooke at the start of this series of illustrations, while the spores are  $13.5 \times 5.5\mu$ .

1881. *R. Pirotta*. Sullo sviluppo della *Peziza Fuckeliana* de By. e della *P. Sclerotiorum* Lib. *Nuovo Giornale Botanico*, 13: 130. See *J. Microsc. Soc.* L, 235-40.

The author describes conditions under which the different stages are formed, but the paper contains no description of the fungus. It is stated incidentally that Brefeld, van Tieghem, Klein, Cornu, Tichomiroff and Schroeter have tried to confirm de Bary's assertion that *Botrytis cinerea* is the conidial stage of *Peziza fuckeliana*. Pirotta himself collected *Sclerotium echinatum* on fallen vine leaves and obtained *Botrytis cinerea*, and in one case six *Peziza* cups.

1884. *A. de Bary*. *Vergleichende Morphologie und Biologie der Pilze, Mycetoza und Bakterien*. Leipzig.

This is usually referred to as the second edition of de Bary 1868, but actually is largely a new work. There are numerous references in it to a fungus referred to indiscriminately as *Peziza fuckeliana* and *Sclerotinia fuckeliana*. It is stated that conidiophores can arise directly on the mycelium growing from ascospores, and further that germinating conidia produce a mycelium with all the characters and products of the mycelium produced from ascospores, except that it inclines more to the production of conidiophores. Frequently 'abortive conidia' or 'spermatia' are formed, and there is a new illustration of the mode of formation of spermatia in *Peziza fuckeliana* (p. 264, fig. 116).

1886. *A. de Bary*. Ueber einige Sclerotien und Sclerotinien-Krankheiten. *Botan. Zeitg.* 22-27 (p. 377).

It is recorded that *Botrytis cinerea* is found only in association with *Sclerotinia fuckeliana*, and not with other species of *Sclerotinia*.

1887. A. de Bary. *Comparative morphology and biology of the fungi, mycetozoa and bacteria*. Oxford.

The English translation of the 1884 work; it apparently contains no further information.

1889. P. A. Saccardo. *Sylloge fungorum*, VIII, p. 196.

*Sclerotinia fuckeliana*. De Bary. *Morph. Pilz.* p. 30, *Fuck. Symb.* p. 330, Pirotta in *N.G.B.* 1. 1881. p. 130—Stipitata, minuta, patellaris,  $\frac{1}{2}$ -3 mm. lata, 5-10 mm. alta, flavo-brunnea ex Sclerotio echinato Fuck. nascens; ascis teretivulvatis,  $130=12-13$ ; sporidiis monostichis, ovoideis, hyalinis,  $10-11=6-7$ .

Hab. in Sclerotio echinato ad folia putrida Vitis viniferae in Germania, Italia.

Status conidicus est *Botrytis cinerea* Pers.

This entry omits references to de Bary's paper of 1869, but the description given is apparently based on the description and figure in this work with the additional information on the colour of the apothecium, which does not seem to have been mentioned by de Bary.

1890. W. Zopf. *Die Pilze*, p. 472.

This work is remarkable for republishing 'Figur 2' from de Bary's 1869 paper and is also unique in citing this paper for the description of *Sclerotinia fuckeliana* instead of the book of 1866. The magnification of the ascus is stated to be ' $\times 300$ ' while in de Bary's paper it is given as ' $\times 390$ ', and this would make the ascus about  $170\mu$  long, and the spores about  $16 \times 5.5\mu$ . From examination of the type specimen Zopf's magnification is evidently a misprint.

1896. H. Rehm. in Rabenhorst's *Kryptogamen Flora*, 1, 3, p. 811.

The descriptions previously cited are apparently all based on de Bary's account and figure, but Rehm appears to have re-examined the fungus and to have based his description on new observations, and as a result his account differs in some respects from de Bary's. He says:

5408: *Scl. Fuckeliana* (De Bary).

Synon.: *Peziza Fuckeliana* De Bary (*Morph. Phys. der Pilze*, p. 30, fig. 12, p. 238).

*Sclerotinia Fuckeliana* Fuckel (*Symb. myc.* p. 330).

Exsicc.: Fuckel, *Fungi rhen.* 1478 (Sclerotium).

Apothecien meist einzeln, aus einem in dem Blattnerve gebildeten, später freien, länglichen oder runden, oft unregelmässigen, 2-4 Millim. langen, 1-2.5 Millim. breiten und dicken, aussen schwarzen, zuletzt glänzenden, feinwarzigen Sclerotium sich entwickelnd, zuerst kuglig geschlossen, dann kelchförmig, rundlich sich öffnend und die zuletzt flach schüsselförmige, zart berandete Fruchtscheibe entblössend, mit einem cylindrischen, geraden oder gebogenen 2-10 Millim. langen, 0.1 Millim. breiten Stiel, aussen glatt, schwach bräunlich, trocken verbogen, 0.2-0.5 Millim. breit, wachsartig. Schläuche cylindrisch, oben abgerundet,  $100-120\mu$  lang,  $9-12\mu$  breit, 8 sporig. Sporen länglich-elliptisch, stumpf,

einzellig mit je einem kleinen Oeltropfen in der Ecke, farblos,  $9-11\mu$  lang,  $5-6\mu$  breit, einreihig liegend. Paraphysen fädig, septiert, nach oben allmählich  $5\mu$  breit, farblos. Gehäuse prosenchymatisch, schwach bräunlich. Jod bläut den Schlauchporus.

An faulenden Blättern des Weinstockes im Rheingau, bei Halle (Winter), Grünberg in Schlesien (Schröter).

In the course of a long discussion of *Sclerotinia fuckeliana* Rehm adds: 'Die Beschreibung geschah nach den in meinem Besitz befindlichen, aus dem *Sclerotium echinatum*, wie es Fuckel l.c. nannte, durch Cultur erzeugenen Exemplaren Winter's.'

1902. Annie Lorrain Smith and Carleton Rea. Fungi New to Britain. *Trans. Brit. mycol. Soc.* xxxv.

Recording a specimen grown from sclerotia of a fungus causing a disease of gooseberry bushes in Hereford, April 1902, the authors translate and slightly simplify Rehm's description quoted above. Their specimen is in Herb. Mus. Brit.

1903. Annie Lorrain Smith. A disease of the gooseberry. *J. Bot.* xli, 19-23.

This gives a further account of the same outbreak of *Botrytis* on a plantation of seven-year-old gooseberries in the Hereford district. In addition to conidia  $8-11 \times 4-6\mu$ , 'a *Peziza* also grew from one of the sclerotia in the damp chamber'. This specimen was said to resemble *Sclerotinia fuckeliana*; ascospores remaining in the ascus were oval and measured  $10-12 \times 6\mu$ .

1905. Gy. de Istvanffi. Études microbiologiques et mycologiques sur le rot gris de la vigne (*Botrytis cinerea*-*Sclerotinia Fuckeliana*). *Ann. de l'Inst. Cent. Ampélogique Roy. Hongrois*, III, 184-360, figs. 1-211.

Istvanffi made a thorough re-examination of sclerotial, conidial and apothecial stages, and described the development of all stages in detail. The asci are stated to be only  $60 \times 6\mu$ , containing from 5-8 spores measuring  $9 \times 4\mu$ , apparently described from microtome sections in paraffin wax stained with Heidenhain's haematoxylin. Apparently apothecia were not obtained from sclerotia grown in pure culture.

1911. P.A. Saccardo. *Sylloge fungorum*, xx. Index Iconium Fungorum, p. 764.

Under '(*Sclerotinia*) *Fuckeliana* de Bary', Saccardo lists twenty-two sources of illustrations, but the figure from de Bary's papers of 1869 and 1872, copied by Zopf 1890 and reproduced here, is omitted.

1927. Said Kharbush. Évolution nucléaire du *Sclerotinia Fuckeliana* de Bary. *Bull. Soc. Bot. de France*, LXXIV, 257-67.

Kharbush states that in artificial culture some sclerotia give sterile apothecia of *Sclerotinia*, others tufts of *Botrytis cinerea* conidiophores. A cytological study was made of fertile apothecia and ascospores were found to be oval or oblong-elliptical,  $9 \times 4\mu$ , and on germination they gave a mycelium producing *Botrytis* conidiophores and sclerotia.

1945. H. H. Whetzel. A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate Discomycetes. *Mycologia*, xxxvii, 648-714.

In this posthumous work the editor, Dr H. M. Fitzpatrick, records that in 1930 Whetzel collected a single apothecium growing from a sclerotium attached to a grape cane, and that his studies indicated that it was *Sclerotinia fuckeliana*. Ascospores from it gave a culture of the *Botrytis cinerea* type. It is unfortunate that de Bary's specimens were not available to Whetzel for use in his studies, because, as Fitzpatrick points out, Whetzel expected to designate *Sclerotinia fuckeliana* as the type species of his new genus *Botryotinia*. The species chosen as the type of the genus was *B. convoluta* Drayton, and the new combinations *B. fuckeliana*, *B. porri* and *B. ricini* were established.

*de Bary's specimens of Peziza Fuckeliana*

The specimens of *P. fuckeliana* in de Bary's collection of slides of cryptogams in the British Museum (Natural History) consist of sections mounted in glycerine and labelled in de Bary's handwriting.

(1) '*Peziza Fuckeliana* 22/10/64.' This slide contains three vertical sections through an apothecium and stalk. The most complete section is illustrated in Pl. I, fig. 1, and shows a disk 1.8 mm. diam., with a hymenial layer approximately  $140\mu$  thick. The stem is cut obliquely and is about 0.6 mm. wide at the top. The fine hairs formed of single hyphae, which clothe the stem, are visible in some places. These hairs are shown at higher magnification in Pl. III, fig. 1.

Pl. II, fig. 1 shows the right-hand margin of the apothecium at a higher magnification. The bi-guttulate ascospores, approximately  $8.5-10 \times 3.5-4\mu$  are clearly shown.

Pl. II, fig. 2 illustrates a portion of one of the other sections on this slide where the layer of asci has been squashed and shows paraphyses.

(2) '*Sclerotium peziza fuckeliana* (Vitis) Fbg [= Freiburg?] Febr. 62. *Peziza*, Sept. 62.' This slide contains three transverse sections through sclerotia. One of these is illustrated in Pl. III, fig. 3, and traces of xylem of the host plant can be seen embedded in the sclerotium.

In addition to these earlier sections of sclerotia, there are six other slides of sclerotia labelled: '*Peziza fuckeliana* 1/8/82.'

(3) '*Peziza fuckeliana*. Spermatium? cult .9/82.' A typical portion of this slide is illustrated in Pl. III, fig. 2, and shows hyphae, spermodochia and spermatia. Probably, this slide was used in making the drawing for fig. 116 of de Bary's book of 1884.

These studies were begun at Seale-Hayne Agricultural College, Newton Abbot, and completed at Rothamsted Experimental Station, Harpenden. Help in tracing literature and slides given by Dr G. R. Bisby, Dr J. Ramsbottom, and Dr R. Eric Taylor is gratefully acknowledged. The writer also wishes to thank the Trustees of the British Museum (Natural History) for permission to publish the photographs, Pls. I-III.

## SUMMARY

Interest in *Sclerotinia fuckeliana*, described by de Bary as the perfect stage of *Botrytis cinerea* on vine, is revived by the production of apothecia in pure cultures by Drayton and Groves. The identification of these apothecia has been hindered by lack of information on de Bary's fungus, the reference usually cited as a description being a *nomen nudum*.

The various descriptions of this fungus are cited, including a detailed account and figure by de Bary, which appear hitherto to have been overlooked. Photographs are also given of de Bary's slide of *Sclerotinia fuckeliana* from the British Museum (Natural History), which evidently constitutes the type specimen. The information now available should help to determine the position of apothecia produced in pure culture.

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## EXPLANATION OF PLATES

*Label on Slide.* 'Peziza fuckeliana 22/10/64.' (Three vertical sections through apothecium, mounted in glycerine.)

## PLATE I

Fig. 1. General structure of apothecium.  $\times 83$ .

## PLATE II

Fig. 1. Margin of same apothecium showing asci with 8 biguttulate ascospores.  $\times 380$ .

Fig. 2. Portion of another section which has been squashed, showing paraphyses.  $\times 380$ .

## PLATE III

Fig. 1. Hairs on stalk of apothecium (just visible on right-hand side of Pl. I, fig. 1).  $\times 380$ .

*Label on Slide.* 'Peziza fuckeliana. Spermatium? cult. 9/82.'

Fig. 2. Groups of microconidial (spermatial) sporodochia.  $\times 380$ .

*Label on Slide.* 'Sclerotium pezizae fuckeliana (Vitis). Fbg. 62. Peziza, Sept. 62.'

Fig. 3. Transverse section of sclerotium with parts of host plant tissue, and traces of xylem embedded in sclerotium.  $\times 195$ .



Fig. 1



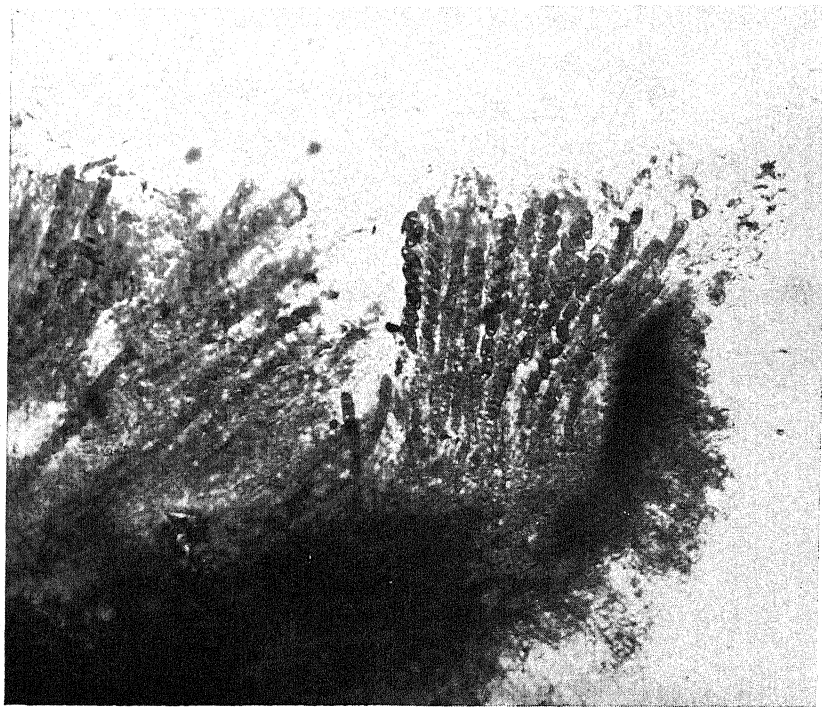


Fig. 1

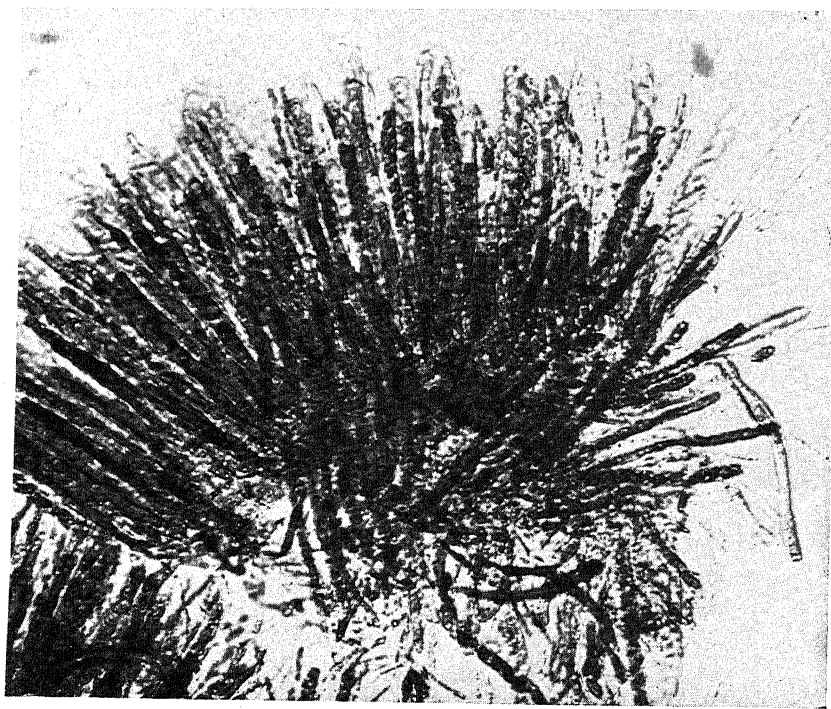


Fig. 2



Fig. 1



Fig. 2

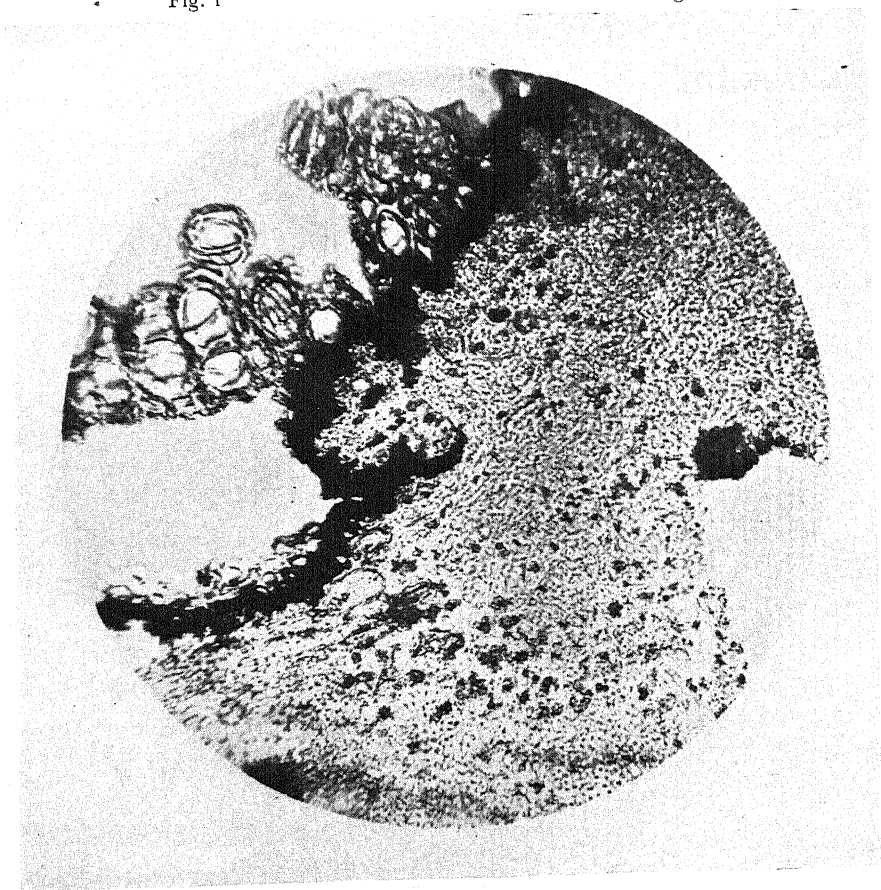
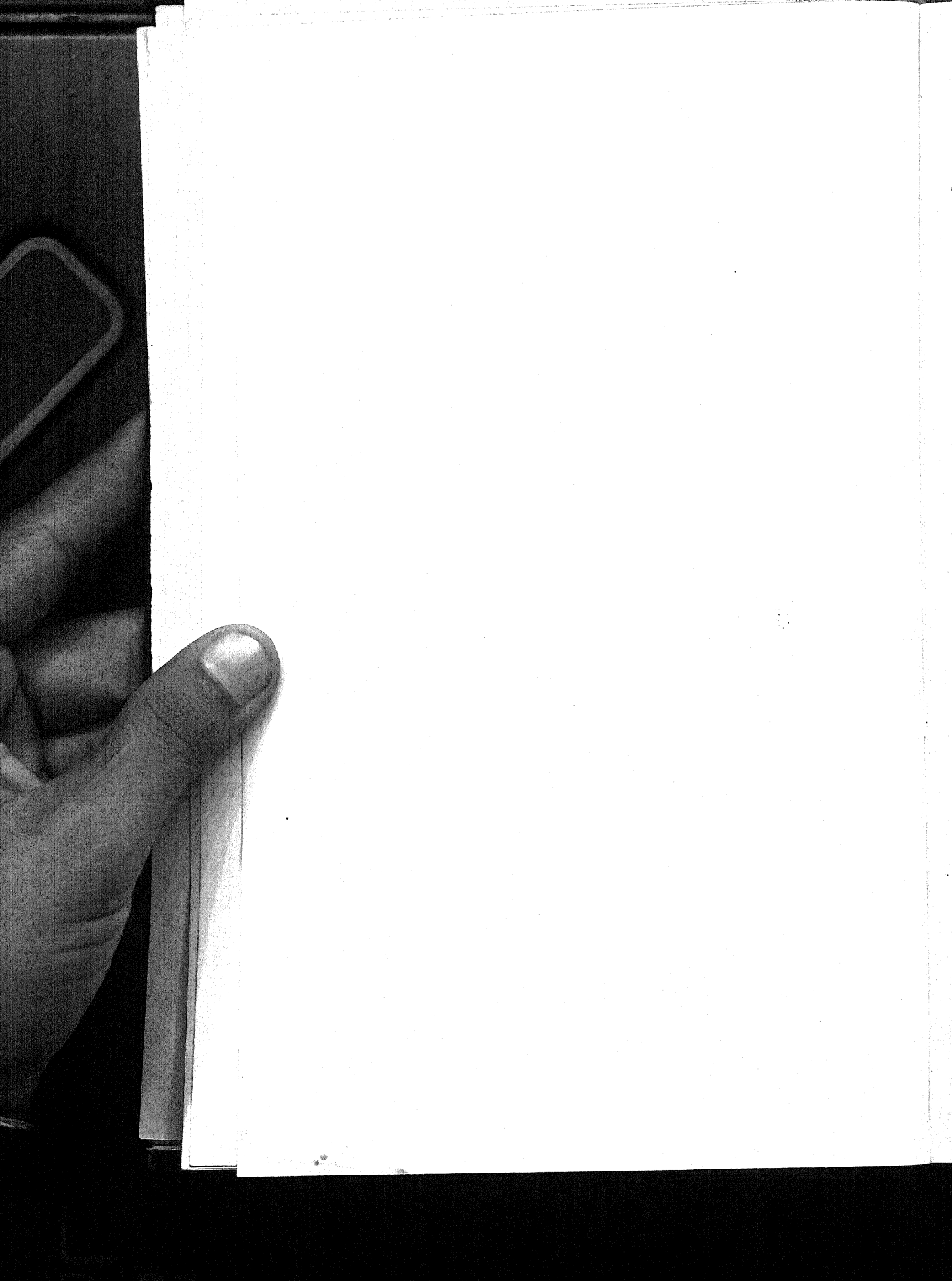


Fig. 3





# THE OPERATION OF THE PUFF-BALL MECHANISM OF *LYCOPERDON PERLATUM* BY RAINDROPS SHOWN BY ULTRA-HIGH-SPEED SCHLIEREN CINEMATOGRAPHY

By P. H. GREGORY

*Agricultural Research Council Research Officer at Rothamsted Experimental Station, Harpenden, Herts*

(With Plate IV and 1 Text-figure)

On blowing across the top of a fruit body of *Lycoperdon perlatum* it is easy to evoke a cloud of spores, but the velocity of the air stream under these conditions is high. Preliminary tests in a small wind tunnel indicated that wind of ordinary speeds is not very effective in removing spores through the apical mouth. The puff-ball mechanism can, of course, be operated mechanically by animals, but emptied peridia are found in places where the number of blows received from animals is unlikely to be sufficient to account for discharge. Other natural agencies were therefore sought, which might operate the puff-ball mechanism in those species such as *L. perlatum*, *L. pyriforme* and *L. echinatum* which dehisce by a central apical pore\*. The studies by Buller (1942) on the splash-cup mechanism of the Nidulariaceae (apparently first discovered by Martin, 1927) suggested that raindrops might also be capable of operating the puff-ball mechanism.

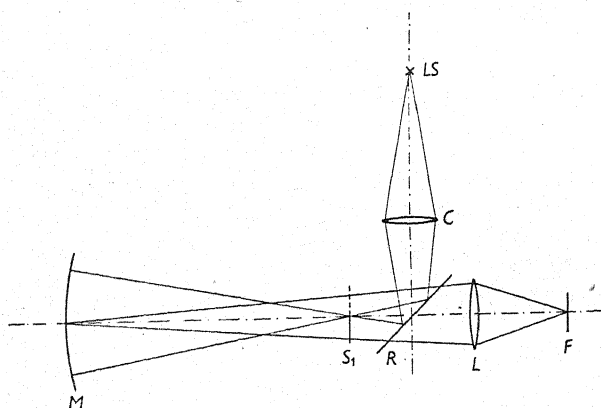
Drops of water were released from a pipette placed over a mature fruit body of *L. perlatum* in the laboratory. Impact of the drop with the papery, flattened top of the peridium, to the side of the ostiole, was instantly followed by the ejection of a small puff of spores to a height of two or three centimetres. Under laboratory conditions vortex rings were sometimes formed. Observations out of doors during rain showed that the mechanism was also operated by raindrops under natural conditions. Fine misty rain did not produce a puff visible in dull diffuse daylight, but thunder rain was excellent. Rain-drip from leaves of trees during fine rain consists of relatively large drops which made the puff-ball 'smoke' as though on fire. *L. perlatum* var. *lacunosum*, *L. pyriforme* and *Geaster umbilicatus*, were also easily operated by raindrops.

The mode of action of the drop was analysed by ultra-high-speed Schlieren cinematography, using a large mature fruit body of *L. perlatum* collected on Harpenden Common in November 1944. The camera used was the Kodak Type III high-speed cine camera described by Jones (1944), fitted with a tuning fork controlled time base which photographs time

\* In such forms as *L. saccatum* and *L. giganteum* the endoperidium peels away at maturity exposing the gleba directly to the action of wind and rain. Spore dispersal in this type has been well described by Falck (1909) in a species referred to as '*L. pyriforme*' but probably identical with our *L. saccatum*.

marks on the edge of the film at 0.001 sec. intervals (Eyles, 1943). The optical system is illustrated in Text-fig. 1, and is similar to that described originally by Taylor and Waldram (1933).

The concave spherical mirror condenser  $M$  is 12 in. in diameter and its surface is optically worked to a high degree of accuracy and sputtered with aluminium to give it high reflectance. An image of the 500 W. compact source high-pressure mercury vapour lamp  $LS$  is thrown by the condenser  $C$ , corrected for spherical aberration, from the surface of the plane mirror  $R$  to the centre of curvature of the mirror  $M$ . The mirror  $R$  is rhodium-plated to reflect about 50 % of the incident light. From the centre of curvature of the concave mirror  $M$ , the light proceeds to its surface and is reflected back along its own path through the plane mirror  $R$  into the



Text-fig. 1.

camera lens  $L$ , forming an image of the concave mirror  $M$  at its focal plane  $F$ , which is the plane of the film in the camera. In this way, an extended field of uniform illumination of high brightness is produced. The brightness is, in fact, equal to that of the surface of the light source itself, reduced only by the reflexion losses at the surfaces of the optical system. The photographs taken under these conditions are therefore shadow photographs of the subject placed close to and in front of the concave mirror  $M$ .

The sensitivity of the optical system described above can be increased by reducing the effective size of the light source  $LS$ . This is conveniently carried out by introducing a knife-edged diaphragm  $S_1$  at the centre of curvature of the concave mirror  $M$ . An image of this diaphragm is thus formed at the same point by reflexion from the concave mirror  $M$ . If the position of the knife edge  $S_1$  is adjusted properly, it can be made to form a fine parallel slit with its own image, reducing effectively the size of the light source  $LS$  in a horizontal plane and thereby increasing the resolving power of the system. This so-called 'Schlieren' system of illumination can be made so sensitive that small variations in the refractive index of the air

or other transparent media in front of the concave mirror *M* will be made visible as shadows in the focal plane of the camera lens *L*. In the case of the photography of spore clouds with which we are dealing, it is doubtful whether the system was adjusted to the high sensitivity required to show up eddies in the air disturbed by ejection of the spores. The spore clouds were, however, effectively larger on the record when photographed with the 'Schlieren' or knife-edged diaphragm in place, because of the increased resolving power of the optical system under these conditions which would make it possible to record parts of the cloud containing relatively few spores.

For calibration a series of photographs was first obtained with a graticule with divisions 1 cm. apart, made on a photographic plate, superimposed in front of the concave mirror. Later series were made without the graticule and with the knife-edge diaphragm in position. Examples of these are shown in Pl. IV, figs. 1-9.

Analysis of a number of runs, made at from 600 to 1770 pictures/sec., shows that the water drops. of 5.0-5.2 mm. diameter, had a velocity immediately before impact of 440-470 cm./sec. For approximately 0.002 sec. after impact the only visible change (not shown in Pl. IV, fig. 3 owing to high contrast) was the development of the splash in the form of the usual corona with radial arms. At 0.003 sec. a puff of spores began to emerge from the ostiole, reaching a height of 0.5 cm. in from 0.004 to 0.008 sec., and 1 cm. in from 0.01 to 0.015 sec. The initial velocity of the puff was from 50 to 400 cm./sec. The cloud slowed down on reaching a height of about 1.5-2.0 cm. at about 0.02-0.04 sec. after impact. When the maximum height was reached the cloud was diffused and carried away by air currents.

No observable deformation of the peridium occurred during or after impact. Presumably, however, the thin surface of the peridium is momentarily invaginated against the supporting layer of the capillitium, leading to the ejection of air and spores through the ostiole. The original shape of the peridium is apparently restored by pressure of the capillitium.

Natural raindrops vary from about 0.2 to 5.0 mm. diameter (Simpson, 1941), and drops of different size have different terminal velocities and therefore strike the peridium with different kinetic energies. The minimum energy required to operate the mechanism was tested in the laboratory with a beam of light directed horizontally across the top of the peridium to show the puff of spores clearly. It was not convenient to produce drops of less than 2.5 mm. diameter, and instead of using various sized drops at their terminal velocity, a standard sized drop was released from different heights. By finding the minimum height required by the drop to gain sufficient velocity to operate the puff-ball, the minimum kinetic energy required can be calculated. An estimate can then be made of the size of the minimum natural raindrop, falling at terminal velocity, required to operate the puff-ball. The kinetic energy of the drop is given by  $\frac{1}{2}mv^2$  (where *m* = mass in g., *v* = velocity in cm.).

It was found that drops 2.5 mm. diameter would operate the fruit body of *L. perlatum* when falling from a height of 5-7 cm. or more, corresponding to a kinetic energy of about 40-60 ergs. Observed terminal velocities of raindrops of various sizes are given by Lenard (1904), and somewhat

higher velocities were reported by Ellison (1944). Calculations from Lenard's data show that a drop of 1 mm. diameter would have a kinetic energy of about 48 ergs and so would be about the minimum size required to operate the mechanism. Lenard found that in ordinary general rain in Switzerland, about 25 % of the drops were 1 mm. or more in diameter, and 42 % raindrops of all types examined were in this category. Laws and Parsons (1944) give somewhat similar data from the United States, while Kelkar (1945) from Poona reported the following proportions of raindrops of 1 mm. diameter and over: drizzle, 0.4; light shower, 18.8; moderate shower, 69.2; heavy shower, 90.6; very heavy shower, 35.1 %. From Lenard's data the number of drops striking 1 sq.cm. of horizontal surface during the fall of 1 cm. of 'ordinary rain' would total about 9000, one-quarter of which would be capable of operating the mechanism. A large fruit body of *L. perlatum* with a horizontal surface of 10 sq.cm. might be operated over a quarter of a million times during a year's exposure in a locality with a rainfall of 100 cm. (39 in.).

The number of spores emitted will obviously depend on a number of factors, including: kinetic energy of the drop, position of hit and deformation of the peridium, size of the puff-ball and number of spores remaining. In order to get some indication of the number of spores ejected as a result of raindrop impact a typical fruit body of *L. perlatum* of approximately 2 cm. diameter, collected near Harpenden, in November 1944, was chosen for test. The puff-ball was anchored at the bottom of a deep beaker whose mouth was covered by a card with a central hole through which drops of water of approximately 4.2 mm. diameter (0.038 g.) were allowed to fall on the top of the moistened peridium. In each test ten drops were allowed to strike the peridium, and after allowing the spores to settle, the floor and sides of the beaker were washed out with water containing a wetting agent. The number of spores liberated was then estimated from samples in a haemocytometer slide.

Two series of tests were carried out with drops falling from each of two heights. The mean number ejected with a drop falling 43 cm. was  $0.99 \times 10^6$  spores, and with a drop falling 130 cm.,  $15.6 \times 10^6$  spores. The total number of spores remaining in the puff-ball was estimated as  $4460 \times 10^6$ .

The outside of the mature peridium is easily wetted by rain. When wet it darkens, swells and becomes more pliable. Wetting of the endoperidium does not hinder puffing, as the wall is waterproof and the spores remain dry. In one test a fruit body of *L. perlatum* was immersed in water to just below the ostiole and would still puff freely when removed and tested after three days.

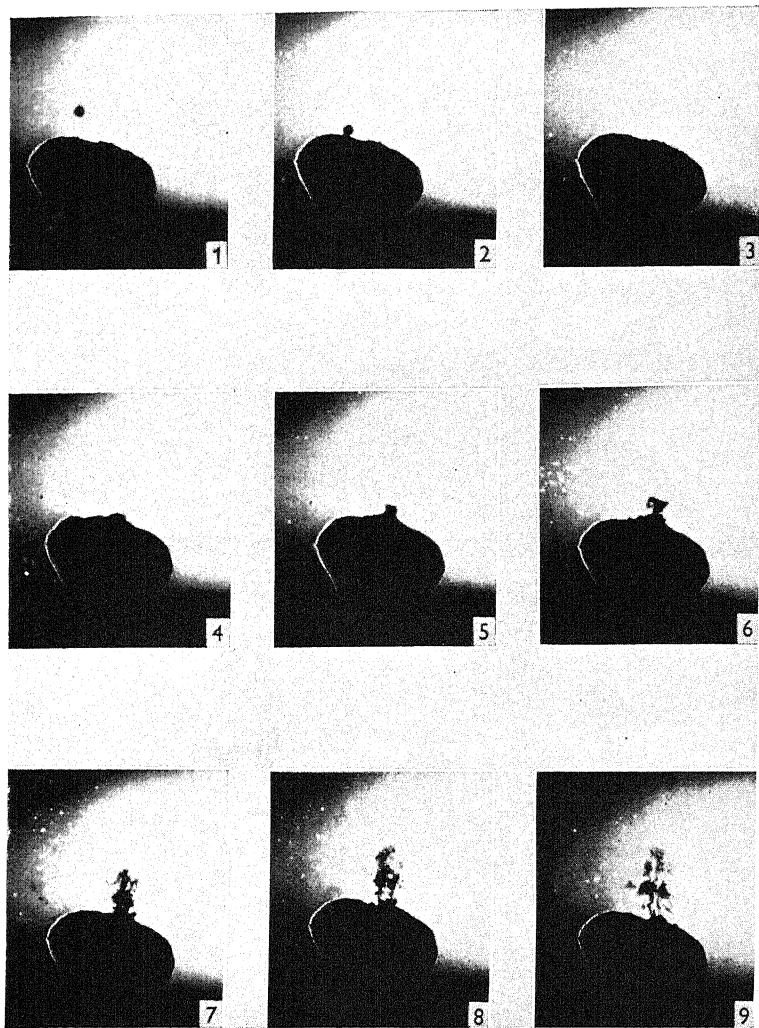
Raindrop impact must therefore be one of the most efficient agents in liberating spores from the *L. perlatum* type of puff-ball. The relative importance of wind and raindrop impact awaits investigation.

#### SUMMARY

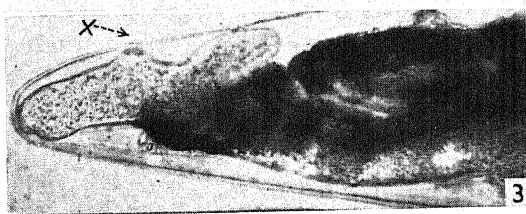
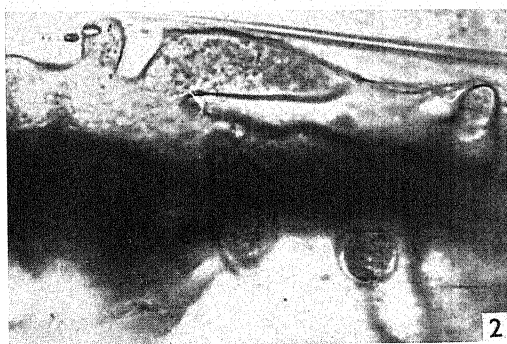
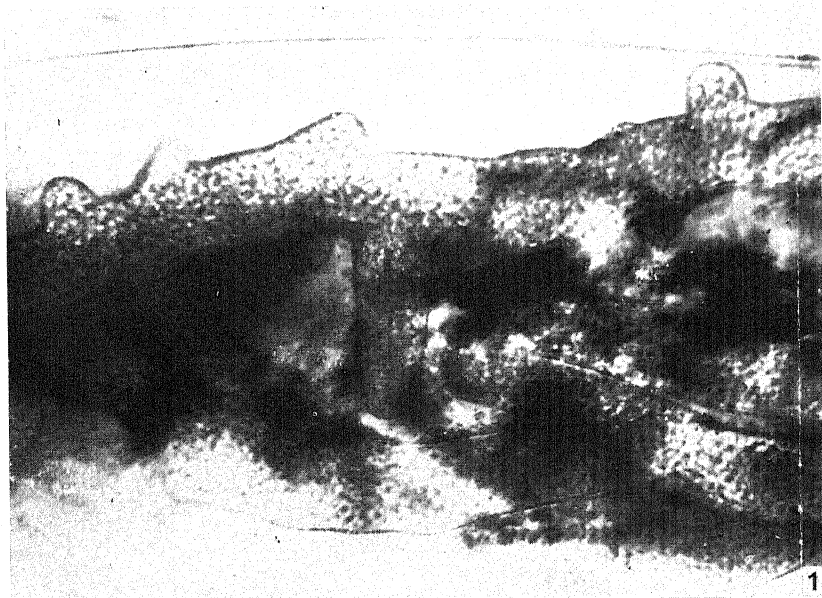
Spore discharge from puff-balls of the *Lycoperdon perlatum* type can be brought about by impact of water drops with the flattened papery top of the endoperidium. Raindrops of 1 mm. diameter or over, and rain-drip







Figs. 1-9







from trees are adequate to operate the mechanism. Analysis of the operation by ultra-high-speed photographs shows that the puff reaches a height of a centimetre in approximately one-hundredth of a second after impact. The velocity of the puff on emerging from the ostiole is of the order of 100 cm./sec.

The endoperidium of *L. perlatum* is normally water-proof and ejection continues under humid conditions. Estimates, made from meteorological data, show that a fruit body must be operated many thousands of times in a season. It is concluded that raindrop impact is efficient in liberating spores from *L. perlatum*.

These observations were incidental to studies on the dispersal of plant pathogens. The photographs were possible only because of the resources made available by the Kodak Research Laboratories, Harrow. In particular the writer is indebted for the collaboration of Mr E. D. Eyles who suggested and successfully carried out the analysis of the spore discharge mechanism by ultra-high-speed Schlieren cinematography.

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#### EXPLANATION OF PLATE IV

Stills from ultra-high-speed film showing impact of water drop with fruit body of *Lycoperdon perlatum* ( $\frac{1}{4}$  of natural size).

- Fig. 1. Drop 5.0 mm. diameter falling with velocity of 440 cm./sec. Time: -0.00167 sec.
- Fig. 2. Impact of drop on peridium to left of ostiole. Time: zero.
- Fig. 3. After impact but before emergence of puff (splash corona not shown in print). Time: +0.00167 sec.
- Fig. 4. Puff of spores beginning to emerge through ostiole with velocity of 140 cm./sec. Time: +0.00334 sec.
- Fig. 5. Stages in diffusion of puff. Time: +0.0050 sec.
- Fig. 6. Time: +0.0067 sec.
- Fig. 7. Time: +0.0020 sec.
- Fig. 8. Time: +0.0032 sec.
- Fig. 9. Time: +0.0046 sec.

(Accepted for publication 2 June 1947)

## STUDIES ON BRITISH CHYTRIDS

IV. *CHYTRIOMYCES TABELLARIAE* (SCHRÖTER) N.COMB.  
PARASITIZED BY *SEPTOSPERMA ANOMALUM*  
(COUCH) WHIFFEN

BY HILDA M. CANTER, PH.D.

*The Freshwater Biological Association, Wray Castle, Ambleside, and  
Department of Botany, Birkbeck College, University of London*

(With 3 Text-figures)

A fungus believed to be *Phlyctidium tabellariae* Schröter (1897) was found parasitizing *Tabellaria flocculosa* (Roth) Kütz in a temporary mud pool bordering the northern edge of Blelham Tarn bog near Wray Castle, during January 1947 and three months later on *T. fenestrata* (Lyngbye) Kützing in the plankton of Crummock Water, Buttermere and Bassenthwaite in the English Lake District. Specimens on *T. fenestrata* were scarce and the following description of the chytrid is based entirely on material collected from Blelham bog. After the latter material had been kept in the laboratory for a week, *Chytriomycetes tabellariae* itself was attacked by the hyperparasite *Septosperma anomalum* (Couch) Whiffen.

I. *Chytriomycetes tabellariae* (Schröter) n.comb.

The spherical, uniguttulate zoospore settles on a host cell, encysts, and by the development of an extramatrical germ tube of varying length (up to  $13\mu$  long) is carried above the surface of the diatom (Fig. 1*c-e, g, j*). In some specimens this extramatrical portion is difficult to observe, and in a few it appears to be absent. The germ tube having entered the diatom cell forms a little branched rhizoidal system of limited extent, which does not taper (Fig. 2*a-c*), and is usually only visible after staining. It is almost certain that *C. tabellariae* shows a similar type of development to *Chytridium schenkii* (Schenk) Scherffel, *C. appressum* Sparrow, and others, in which a part only of the zoospore wall enlarges to form the sporangium, the remainder persisting as an appendage. In *Chytriomycetes tabellariae* a part of the zoospore grows out to form a more or less oval sporangium, while the unexpanded portion persists as a swelling to which the extramatrical germ tube is attached. This swelling does not thicken as in *Chytridium schenkii* and may possibly represent a part of the already expanded zoospore which failed to develop further when the unilateral expansion began to form. When the sporangium is viewed from above this spherical portion cannot be seen (Fig. 1*i-k*). The changes in the protoplasm during development are similar to those described for the majority of chytrids. Before the sporangium is mature the portion of the wall forming the operculum is well marked (more especially in the larger specimens, Fig. 1*i*). It occupies a position

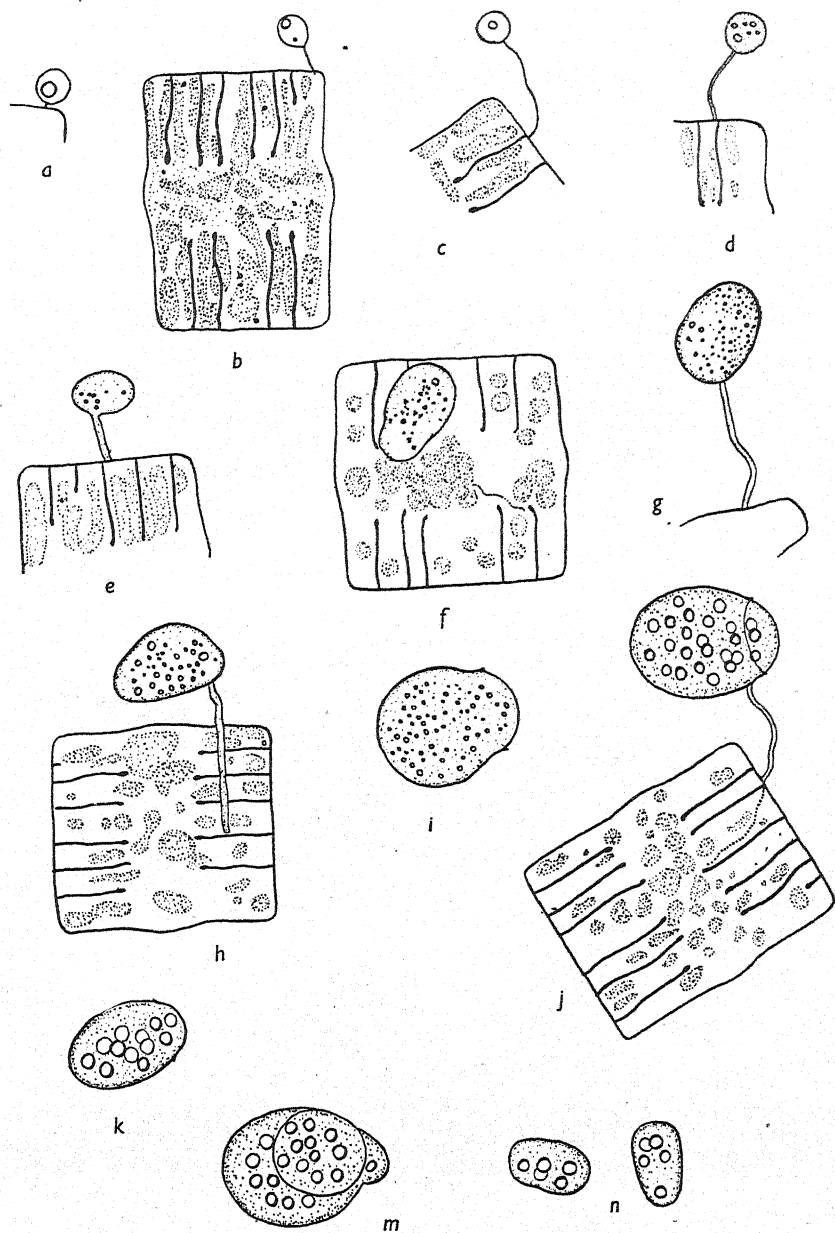


Fig. 1. *Chytridiomycetes tabellariae*. a-h, stages in early development of thallus. i, immature sporangium, operculum clearly visible. j-m, mature sporangia. All  $\times 1400$ .

immediately above the basal spherical portion. The mature sporangia vary in size from  $4.3-9\mu$  high  $\times 7.6-15\mu$  broad and contain from 5 to 30 zoospores according to their size (Fig. 1*n, m*). The actual moment of dehiscence was never observed, and thus it is not known whether the zoospores emerge singly or in a mass. The zoospores are spherical,  $3\mu$  in diameter, with a conspicuous oil globule and single posterior flagellum.

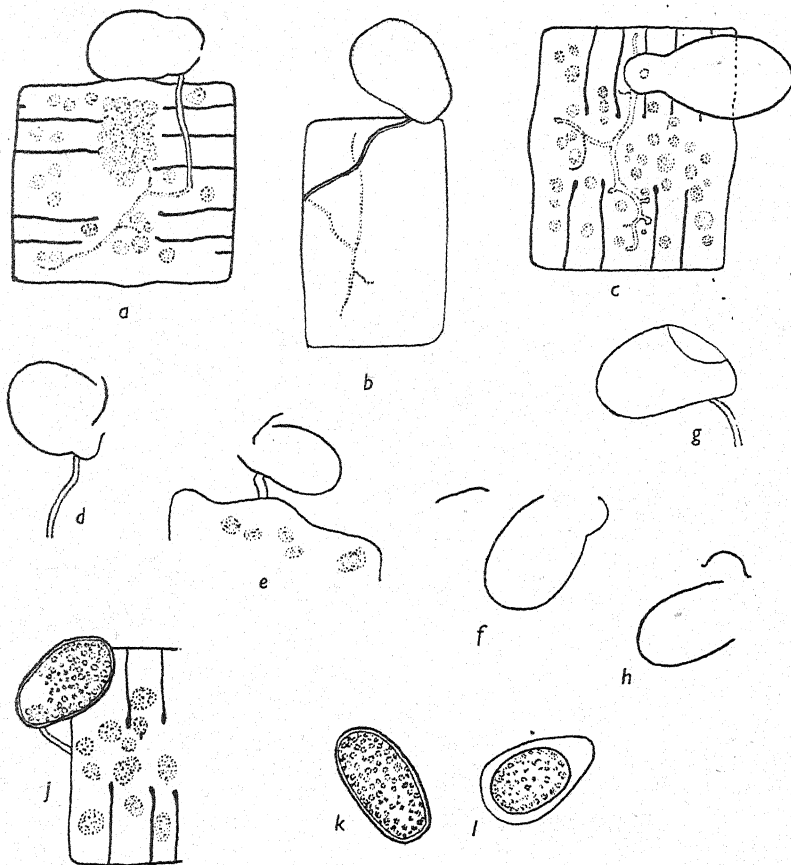


Fig. 2. *Chytriumyces tabellariae*. a-c, stained specimens showing full extent of rhizoidal system. d-h, empty sporangia. j, k, mature resting spores. l, resting spore formed by rounding off of protoplasm of the sporangium. All  $\times 1400$ .

The convex operculum,  $4.3-8\mu$  in diameter, often remains adherent to the empty sporangium or in its vicinity. Owing to the spherical unexpanded portion of the sporangium, the side of the wall from which the germ tube arises is longer than the opposite wall (Fig. 2*d-f*), and it is this longer side which is measured in determining the breadth of the sporangium, while the height is taken in the mid-region.

A few resting spores were found as the material became moribund. They are borne as the sporangia and appear to be asexually formed. The mature resting spore (Fig. 2j, k) is oval,  $12-9.9\mu$  broad  $\times$   $6-5.7\mu$  high, with a thick, smooth wall and it contains numerous small globules. The rhizoidal system is similar to that formed by the sporangium, but the unexpanded portion of the latter was not seen. In some specimens (Fig. 2l) the resting spore seemed to be formed by the rounding off of the protoplasm of the sporangium and the subsequent secretion of a thick wall. Its method of germination is unknown.

*Phlyctidium tabellariae* has not been found, since its original description in 1897 by Schröter, on *Tabellaria fenestrata* (Lyngbye) Kützing var. *asterioneloides* Grunow. Schröter's description of this chytrid is incomplete and his figures are very small. Nevertheless, the British material agrees in the shape and thick-walled nature of the sporangium, in its basal area of dehiscence, point of origin of the extramatrical stalk, as well as in the nature of its host. It is thus suggested that these two organisms are synonymous, but in view of its operculate nature it is necessary to transfer this species to an operculate genus.

At present the operculate series of monocentric chytrids contains one genus, *Chytriumyces*, in which both the zoosporangia and asexually-formed resting spores are extramatrical and formed by enlargement of the zoospore body. There are four species; *C. aureus* and *C. hyalinus* Karling (1945), *C. nodulatus* Haskins (1946) are saprophytes on insect exuviae, grass leaves, etc., while *C. spinosus* Fay (1947) is not markedly chitinophilic and grows more readily on carbohydrate substrata. In general, the sporangia are spherical, with an extensive, much branched rhizoidal system and the subsporangial swelling is not a constant feature in any one species.

It is clear that the fungus on *Tabellaria* differs from members of *Chytriumyces* in the method of development of the sporangium and in the nature of its rhizoidal system. If the operculate nature of *Rhizophyidium echinatum* (Dang) Minden & Fischer, a parasite on *Glenodinium cinctum* is confirmed, then this fungus with its epibiotic sporangia and asexually-formed resting spores, both developed from enlargement of the zoospore, would show affinities with *Chytriumyces tabellariae*. It is proposed to include the fungus here described, which is identified with *Phlyctidium tabellariae*, in the genus *Chytriumyces* and the name becomes, therefore, *C. tabellariae* (Schröter) n.comb.

## II. SEPTOSPERMA ANOMALUM (COUCH) WHIFFEN

*Septosperma anomalum* (*Phlyctidium anomalum*) was originally described by Couch (1932) parasitizing *Phlyctidium bumilleriae* Couch. Later Whiffen (1942) discovered a similar form growing on *Rhizophyidium macrosporum* Karling and in view of the septate nature of the resting spore erected a new genus, *Septosperma*, to include these species, which have only been recorded from America. The British material (Fig. 3a-q) agrees well with *S. anomalum*. The sporangia are sessile, ovoid or ellipsoid,  $6.6-12\mu$  high  $\times$   $3.8-6.1\mu$  broad, and possess a small endobiotic, bulbous disk. Small sporangia produce about eight zoospores while up to thirty are formed in large ones. The zoospores are spherical,  $2.3\mu$  in diameter, with a small oil globule and



posterior flagellum. The resting spores,  $6.6-12.4\mu$  high  $\times$   $3.3-4.3\mu$  broad, agree with those formerly described. It is of interest that the hyperparasite attacks both young (Fig. 3*c*) and mature sporangia, in which the oil globules of the zoospores are delimited (Fig. 3*b*), but never mature resting spores. Infected specimens never reached maturity or as far as is known, liberated zoospores.

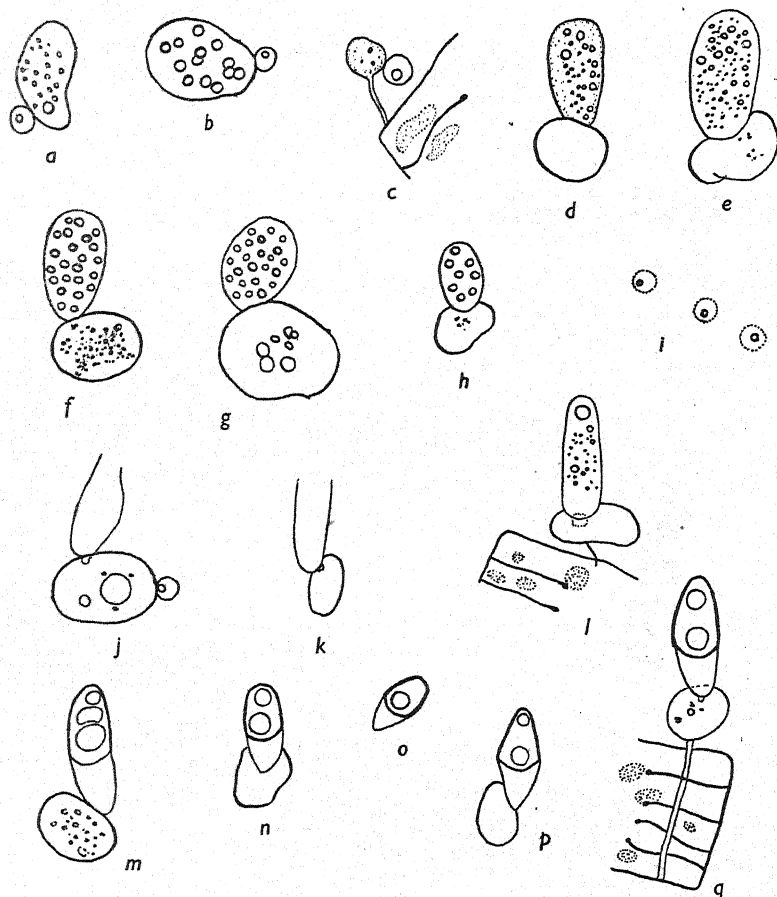


Fig. 3. *Septosperma anomalum*. *a-c*, zoospores attacking sporangia of *Chytrium tabellariae*. *d, e*, immature sporangia. *f-h*, mature sporangia. *i*, zoospores. *j, k*, empty sporangia, rhizoidal disk visible inside host sporangia. *l*, young resting spore. *m-q*, mature resting spores. All  $\times 1400$ .

#### SUMMARY

Two parasitic chytrids, both new records for Great Britain, are described. *Phlyctidium tabellariae* Schröter is found to be operculate and is transferred to the genus *Chytrium* Karling; the name becomes *C. tabellariae* Schröter n.comb. *Septosperma anomalum* (Couch) Whiffen was found parasitizing *C. tabellariae*.

My thanks are due to the Director of the Freshwater Biological Association for the use of a laboratory in which this work was done and to Prof. C. T. Ingold for reading the manuscript.

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## STUDIES ON BRITISH CHYTRIDS

V. ON *OLPIDIUM HYALOTHECAE* SCHERFFEL  
AND *OLPIDIUM UTRICULIFORME* SCHERFFEL

By HILDA M. CANTER

*The Freshwater Biological Laboratory, Wray Castle, Ambleside  
and Department of Botany, Birkbeck College, University of London*

(With Pl. V and 5 Text-figures)

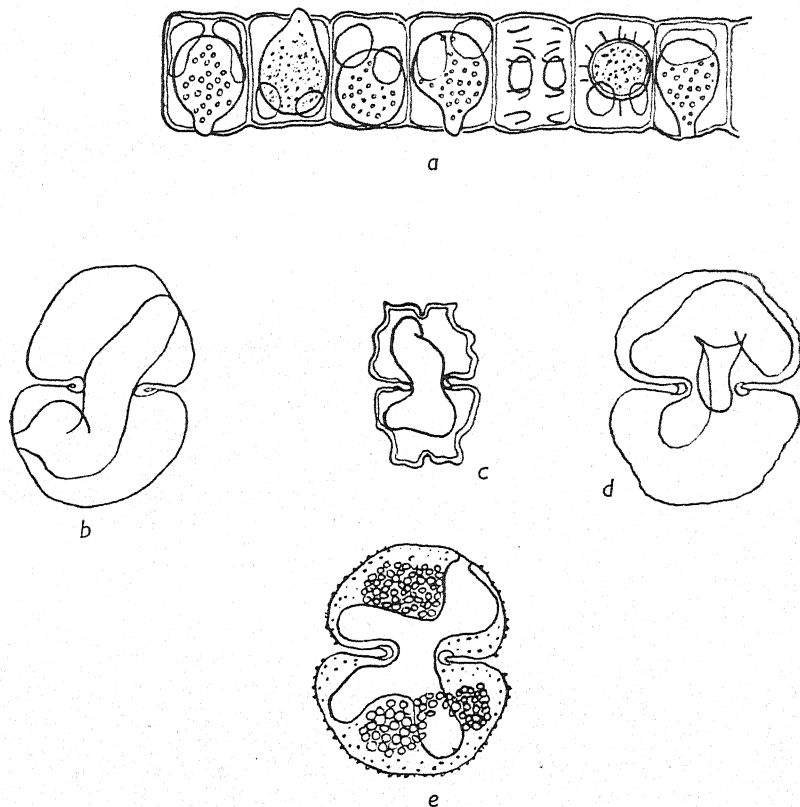
Since *Olpidium hyalothecae* and *O. utriculiforme* were originally described by Scherffel (1926) from Hungary, no further descriptions of them have appeared in the literature. What are considered to be these species were found by me in the bog bordering the northern side of Blenheim Tarn near Wray Castle; *O. hyalothecae* occurred in April and May and *O. utriculiforme* in January 1947. *O. hyalothecae* was again found in April on the shore of Esthwaite Water near Hawkshead.

I. *OLPIDIUM HYALOTHECAE* SCHERFFEL

This chytrid is recorded by Scherffel (1926) parasitizing *Hyalotheca mucosa* (Mert.) Ehrenb. and *H. dissiliens* (Sm.) Bréb., but the British material has so far only been found in the latter.

*Hyalotheca* is usually surrounded by a wide mucilage sheath which was apparently not present in the Hungarian material (Text-fig. 1a). When an alga is surrounded by mucilage it is usual for the chytrid zoospore to encyst on the surface of the mucilage and for the germ tube to grow to the surface of the host cell (e.g. *Dangeardia mammillata* Schröder on *Eudorina elegans*, Canter, 1946). In *Olpidium hyalothecae* this is not so; the zoospore itself penetrates the mucilage until it reaches the host cell. A few specimens were seen in which the zoospore had just begun or had penetrated half way through the mucilage (Text-fig. 2f, h). From the tip of every zoospore settled on the host wall, a faint line can be seen passing to the external surface of the mucilage, either vertically or somewhat diagonally (Text-fig. 2d, f-h). This line indicates the path of the zoospore through the mucilage. The contents of the zoospore pass into the alga while the empty zoospore case and path of penetration remain clearly visible. Very early stages of development of the parasite within the host cell are obscured by the dense contents, and the sporangium is first visible as a spherical, walled body with large scattered oil globules (Text-fig. 2i). Before the sporangia are mature the apex of the neck is filled with mucilage which extends for a short distance outside the cell (Text-fig. 2j, k). The mature sporangia are pear-shaped,  $20-9\mu$  high  $\times$   $13-6.5\mu$  broad, with a short neck which reaches up to the surface of the host wall but never projects beyond. Although the actual moment of dehiscence was never seen, the zoospores

appear to be fully formed within the sporangium. They are spherical,  $3-4\mu$  in diameter with a small posterior oil globule, and a flagellum about  $12\mu$  long. Owing to the presence of the mucilage sheath around the alga

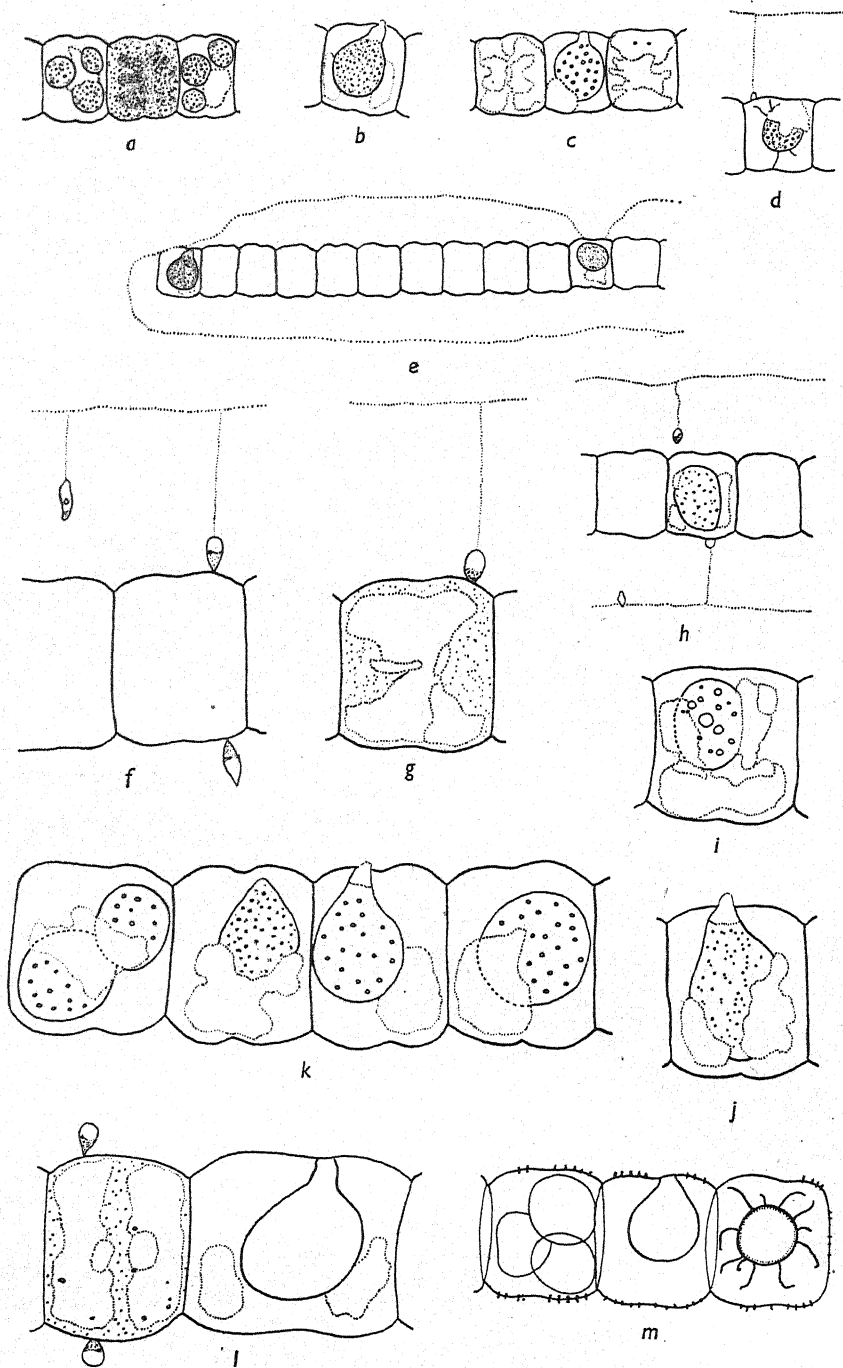


Text-fig. 1. *a. Olpidium hyalothecae* after Scherffel (1926). *b-e, O. utriculiforme* after Scherffel (1926).  $\times 500$ .

it is puzzling to know how the zoospores escape into the external medium. Staining with Indian ink shows that the mucilage in the vicinity of the empty sporangia has disappeared (Text-fig. 2*e*), and the Indian ink particles rapidly enter these sporangia. It would seem that the mucilage does not disappear until the sporangium is mature, for several specimens

#### Legend to Text-fig. 2.

Text-fig. 2. *Olpidium hyalothecae*. *a-c*, stages in development of the sporangium. *d*, resting spore. *e*, part of a *Hyalothecha* filament in Indian ink; note absence of mucilage sheath in region of empty sporangia; the latter are filled with Indian ink particles. *f, g*, zoospores penetrating the mucilage or recently settled on the host wall; the vertical dotted line indicates their path of penetration. *h*, immature sporangium with empty zoospore case and path of penetration clearly visible. *i*, young sporangium. *j*, immature sporangium mucilage papilla well developed. *k, l*, immature, mature and empty sporangia. *m*, empty sporangia and resting spore after staining with Chlorazol black E in lactophenol. *a-d, h*,  $\times 500$ ; *f, g, i-l*,  $\times 1050$ ; *e*,  $\times 300$ ; *m*,  $\times 800$ .



Text-fig. 2.

were observed in which, although the oil globules of the zoospores were delimited, no mucilage had been dissolved.

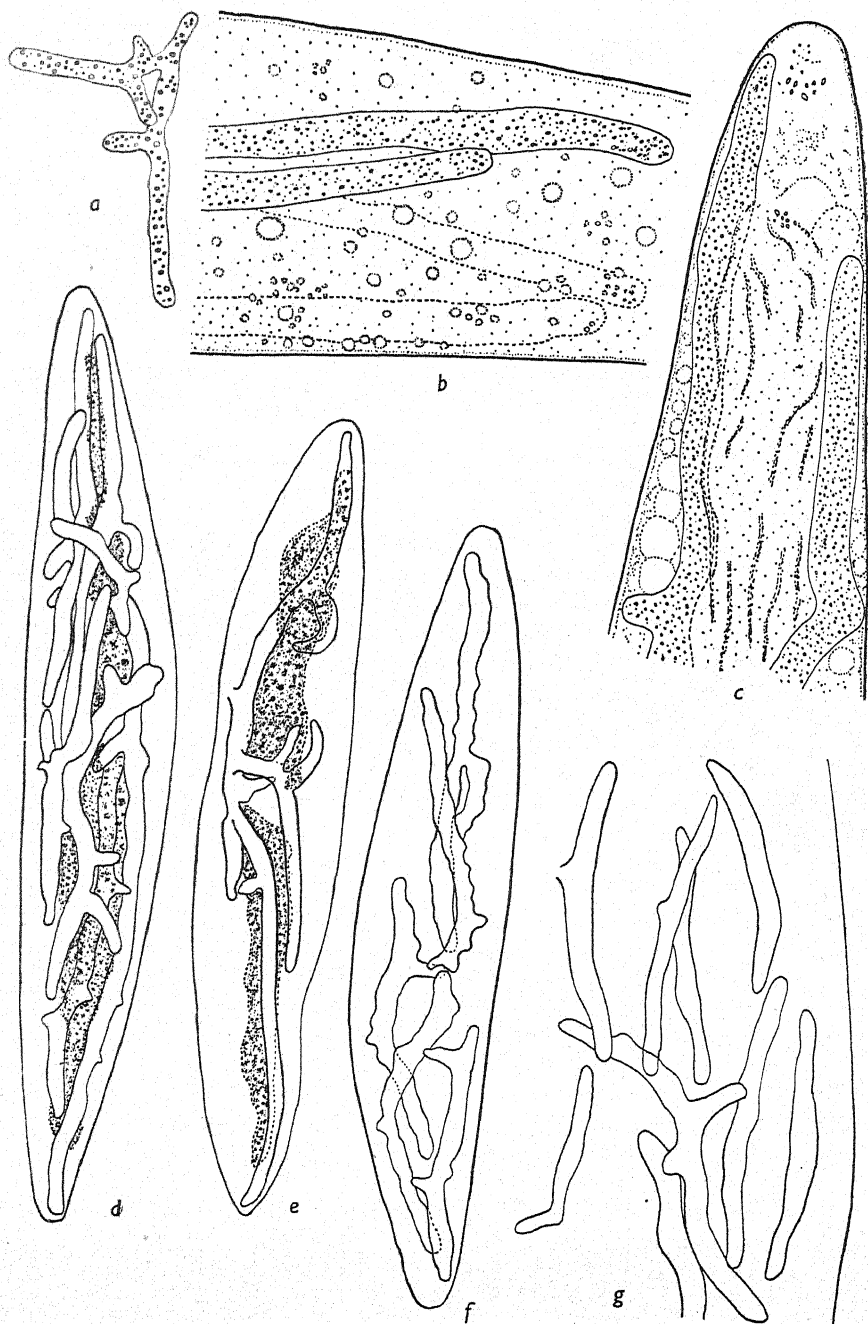
Resting spores were rare, but the few seen agree with those described by Scherffel. They are spherical,  $10\mu$  in diameter, with a smooth wall, the outer surface of which bears a few long processes (Text-fig. 2*m*) which are clearly visible after staining with Chlorazol black E. Their germination was not observed.

## II. *OLPIDIUM UTRICULIFORME* SCHERFFEL

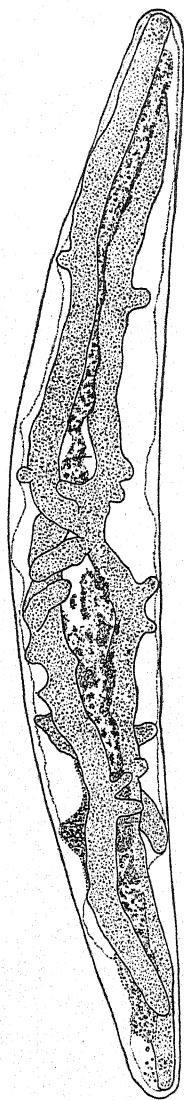
This chytrid (Text-figs. 3-5 and Pl. V, figs. 1-4) occurred as a parasite in *Closterium lunula* Ehrenb., *C. costatum* Corda and *C. dianeae* Ehrenb. Owing to the dense algal contents very young stages in infection were not observed. It is thus unknown whether the whole content of the zoospore passes into the host as a naked mass of protoplasm which soon becomes walled, or whether the zoospore puts forth a germ tube with a delicate wall which subsequently thickens.

The thallus, when first distinguishable, is surrounded by a thin wall and consists of a narrow unbranched tube with hyaline cytoplasm containing large oil globules (Text-fig. 3*a*). This tube elongates, branches, the wall thickens and at maturity the whole forms a single sporangium. The mature thallus is often extensive, consisting of several tubes (up to  $550\mu$  long by  $11-20\mu$  wide) which taper slightly towards their extremities and run parallel to the length of the *Closterium* (Text-fig. 4). Shorter lateral branches are also present. As well as these extensive thalli, small unbranched or little branched forms occur ( $50-90\mu$  long by  $5-8\mu$  wide, Text-fig. 3*f, g*). The number of parasites in a host cell varies from one to twelve and the smaller tend to be formed in cells which already contain a large thallus or where several are crowded together.

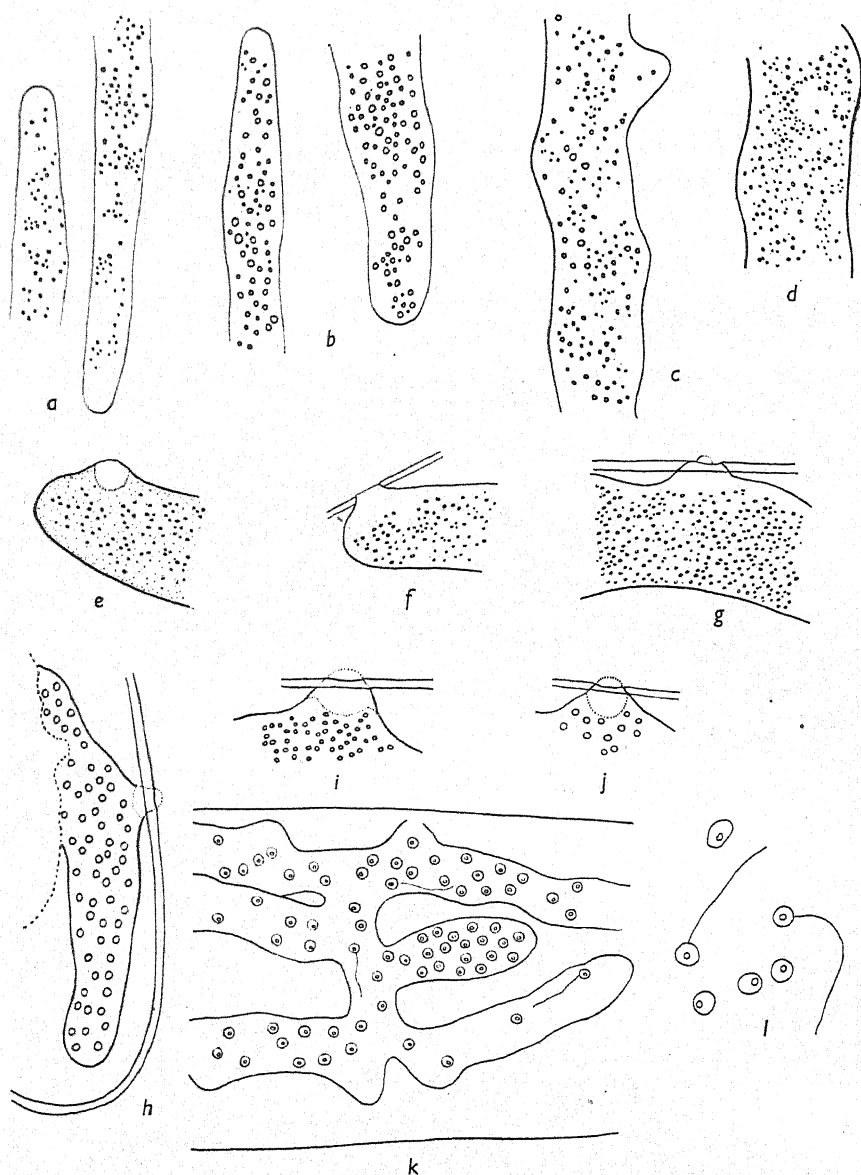
As the parasite grows the host content shrinks, the protoplasm becomes foamy and vacuolate and the periphery is occupied by minute streaming granules. By the time the chytrid is mature the two algal chloroplasts are reduced to brown masses. Young thalli, when stained with chlor-zinc-iodide, give no reaction, while dehiscent sporangia stain faintly purple. The changes in the protoplasm during the development of this fungus are similar to those recorded for the majority of chytrids. At first the fungal content is hyaline with oil globules collected together in certain regions (Text-fig. 5*a, b*). These become smaller and more evenly distributed (Text-fig. 5*d*). Later the protoplasm is granular, with scattered, highly refractive granules (Text-fig. 5*e*) which increase in size to form the oil globules of the zoospores. Before the zoospores are mature certain areas of the thallus adjacent to the *Closterium* wall become gelatinous forming a dully refractive plug which projects slightly beyond the external surface (Text-fig. 5*g-j*). The hundreds of zoospores fully formed within the sporangium are liberated either into the external medium or into the cavity of the host cell. They are spherical,  $2.4-2.8\mu$  in diameter, with a conspicuous oil globule, somewhat lateral in position, and a posterior flagellum  $14-19\mu$  long; they may be amoeboid or exhibit a smooth gliding movement.



Text-fig. 3. *Olpidium utriculiforme*. *a*, very young thallus. *b*, *c*, young sporangia with branches extending along the length of the *Closterium* whose content in (*c*) shows signs of disintegration. *d-f*, host cells containing from one to several sporangia. *g*, small unbranched or little branched sporangia. *a-c*, *g*,  $\times 500$ ; *d-f*,  $\times 185$ .



Text-fig. 4. *Olpidium utriculiforme*. An extensive sporangium in *Closterium lunula* Ehrenb.  $\times 220$ .



Text-fig. 5. *Olpidium utriculiforme*. *a-h*, protoplasmic changes in the sporangium up to the formation of the oil globules of the zoospores; in *e-j* dehiscence papillae are visible. *k*, part of a sporangium with mature zoospores. *l*, zoospores. *k*,  $\times 500$ ; the rest,  $\times 1050$ .

The material of *Olpidium utriculiforme* was extensively attacked by a filamentous fungus (possibly a species of *Pythium*) and disappeared without the formation of resting spores.

Scherffel's figures of *Olpidium utriculiforme* are shown in Text-fig. 1b-e. The form described above agrees in most details with Scherffel's organism, except for the presence of the extensive thalli. The size and degree of branching of the fungus is clearly limited by the host size and whereas Scherffel's form occurred in the smaller desmids such as *Cosmarium botrytis* and *Euastrum* sp., mine was found in large species of *Closterium*. A similar condition exists in *Myzocyttium megastomum* De Wildeman which attacks desmids, where small reduced thalli develop in the smaller algae (Canter, 1947).

Again, *Olpidium utriculiforme* shows a striking resemblance to *Mitochytridium ramosum* Dangeard (1911), but in this form the thallus bears rhizoids. This raises the question to what extent the presence or absence of rhizoids should be used to determine generic distinction. In *Chytridium lagenaria* Schenk pro parte within the same collection I have found thalli with the apophysis bearing an extensive rhizoidal system, whereas in others it is absent. This was also recorded very rarely by Sparrow (1936). It is only when the resting spores of *Mitochytridium* are more definitely known and those of *Olpidium utriculiforme* discovered that the true affinities of these fungi will become clear.

#### SUMMARY

*Olpidium hyalothecae* Scherffel and *O. utriculiforme* Scherffel are recorded for the first time in Great Britain.

My thanks are due to the Director of the Freshwater Biological Association for the use of a laboratory in which this work was carried out and especially to Prof. C. T. Ingold for reading the manuscript.

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#### EXPLANATION OF PLATE V

##### *Olpidium utriculiforme* Scherffel

- Fig. 1. Part of a mature sporangium.  $\times 750$ .  
Fig. 2. Immature sporangium.  $\times 750$ .  
Fig. 3. Immature sporangium with dehiscence papilla visible at X.  $\times 400$ .  
Fig. 4. Empty sporangia.  $\times 360$ .

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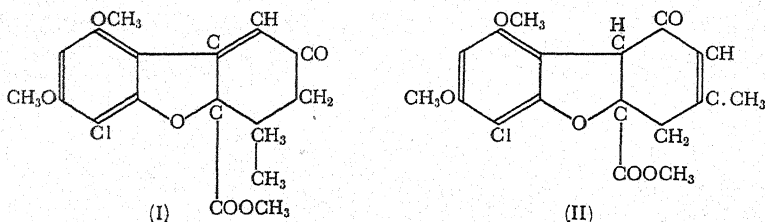


# A SUBSTANCE CAUSING ABNORMAL DEVELOPMENT OF FUNGAL HYPHAE PRODUCED BY *PENICILLIUM JANCZEWSKII* ZAL.

## III. IDENTITY OF 'CURLING FACTOR' WITH GRISEOFULVIN

By P. W. BRIAN, P. J. CURTIS AND H. G. HEMMING  
*Butterwick Research Laboratories, The Frythe, Welwyn, Herts*

In a previous communication (Brian, Curtis & Hemming, 1946) a metabolic product of *Penicillium janczewskii* Zal. was described, which had the unusual property of causing marked morphological abnormalities in the development of *Botrytis* hyphae. This substance, known as 'curling factor', was at first thought to contain only carbon, hydrogen and oxygen and the molecular formula  $C_{20}H_{20}O_9$  was proposed (McGowan, 1946). Subsequent investigation showed that it also contained chlorine (Grove & McGowan, 1947) and was, in fact, chemically identical with griseofulvin ( $C_{17}H_{17}O_6Cl$ ), first isolated by Oxford, Raistrick & Simonart (1939) from the mycelium of *Penicillium griseofulvum* Dierckx. The structural formulae proposed by Oxford *et al.* (I) and by Grove and McGowan (II) are shown below:



The present communication is intended to give the biological evidence for the identity of griseofulvin and 'curling factor' and to present certain other data concerning its production that have arisen from the discovery of this identity.

### BIOLOGICAL CONFIRMATION OF IDENTITY OF 'CURLING FACTOR' WITH GRISEOFULVIN

Solutions (100  $\mu$ g./ml.) of authentic griseofulvin from *P. griseofulvum*, kindly supplied by Prof. Raistrick, and of 'curling factor' from *P. janczewskii* were made up in Weindling solution. These were assayed by a method previously described (Brian *et al.* 1946), *Botrytis allii* being the test organism. Both samples produced the typically stunted and distorted growth and were of equal activity. A further sample of griseofulvin, also supplied by Prof.

Raistrick, which had been obtained from *Penicillium patulum* (Raistrick, 1947) was also assayed, with similar results. These observations, taken in conjunction with the chemical evidence already quoted, establish beyond doubt the identity of 'curling factor' and griseofulvin. Thus, on the evidence at present available, it appears that griseofulvin is produced by at least three different species belonging to three distinct major divisions of the genus *Penicillium*: *P. griseofulvum* Dierckx (Assymetrica-Funiculosa), *P. janczewskii* Zal. (Lanata-Divaricata), *P. patulum* Bain. (Fasciculata).

# ACTIVITY OF CULTURE FILTRATES OF *PENICILLIUM JANCZEWSKII* AND *PENICILLIUM GRISEOFULVUM*

Three strains of *P. griseofulvum* and two strains of *P. janczewskii* were grown on a Czapek-Dox medium, containing 7.5 % of crude glucose, in 100 ml. flasks and samples were taken periodically for assay. The strains used were as follows:

- No. 29. *P. janczewskii*: first obtained in September 1942 as a laboratory contaminant (i.e. before our first isolations from Wareham soil).
- No. 384. *P. janczewskii*: isolated from a cave soil (Read's cavern, Somerset) in 1947.
- No. 374. *P. griseofulvum*: obtained from Prof. Raistrick (LSHTM Catalogue No. P 38)—the strain used for the original isolation of griseofulvin.
- No. 375. *P. griseofulvum*: obtained from Prof. Raistrick (LSHTM Catalogue No. P 68).
- No. 370. *P. griseofulvum*: obtained from Centraalbureau voor Schimmelcultures, Baarn, Holland.

The results of the experiment are shown in Table 1. Both 'curling activity' and inhibition of germination are recorded. In the code used for indicating 'curling activity' the index figure following the letter S denotes the greatest dilution producing marked stunting of the germ-tube and the index figure following the letter C the greatest dilution producing any morphogenetic effect. The figures given under the heading 'inhibition-assay' indicate the greatest dilution completely inhibiting germination.

Table 1. Assays of culture filtrates from *Penicillium griseofulvum* and *Penicillium janczewskii*

Culture No.	Species	'Curling' assay (days growth)				Inhibition assay (days growth)			
		5	9	13	17	5	9	13	17
29	<i>Janczewskii</i>	S <sub>16</sub> C <sub>128</sub>	S <sub>32</sub> C <sub>256</sub>	S <sub>64</sub> C <sub>512</sub>	S <sub>32</sub> C <sub>256</sub>	—	—	—	—
384	<i>Janczewskii</i>	S <sub>8</sub> C <sub>256</sub>	S <sub>8</sub> C <sub>128</sub>	S <sub>16</sub> C <sub>512</sub>	S <sub>16</sub> C <sub>512</sub>	—	—	—	—
374	<i>Griseofulvum</i>	S <sub>8</sub> C <sub>64</sub>	S <sub>64</sub> C <sub>1024</sub>	S <sub>32</sub> C <sub>1024</sub>	S <sub>28</sub> C <sub>1024</sub>	—	—	—	—
375	<i>Griseofulvum</i>	S <sub>0</sub> C <sub>0</sub>	S <sub>64</sub> C <sub>0</sub>	S <sub>64</sub> C <sub>0</sub>	S <sub>64</sub> C <sub>0</sub>	6	24	16	16
370	<i>Griseofulvum</i>	S <sub>0</sub> C <sub>0</sub>	S <sub>0</sub> C <sub>0</sub>	S <sub>2</sub> C <sub>0</sub>	S <sub>4</sub> C <sub>64</sub>	—	—	—	—

Both strains of *P. janczewskii* developed typical 'curling activity'. This now appears to be quite characteristic of this species as all strains examined, from three different sources, have shown the activity. One strain of *P. griseofulvum* (No. 374—Raistrick P. 38) showed similar, possibly higher,

activity. The Baarn strain of *P. griseofulvum* showed a slight activity, developed very late. The remaining strain of *P. griseofulvum* (No. 375—Raistrick P 68), though morphologically very similar to No. 374, showed no 'curling activity' but did appear to produce a fungistatic substance, preventing germination of *Botrytis allii*.

#### GRISEOFULVIN CONTENTS OF MYCELIUM AND CULTURE FILTRATE

Oxford *et al.* (1939) isolated griseofulvin from the mycelium of *Penicillium griseofulvum*. From 3000 g. of mycelium they obtained 50 g. of griseofulvin. To obtain the 3000 g. of mycelium, 259 l. of medium were used, the cultures being incubated for 65–85 days at 30° C. We have extracted 'curling factor' from 11 to 14-day culture filtrates of *P. janczewskii* obtaining yields of 150 mg./l. or about 40 g. from 259 l. It thus appeared probable that by extracting the mycelium as well as the culture filtrate of *P. janczewskii* yields might be approximately doubled. This has in fact been found to be the case as illustrated by the following data for a batch of fifty cultures each on a litre of medium:

Volume of culture filtrate: 50 l.

Weight of griseofulvin extracted from culture filtrate: 8.5 g.

Dry weight of mycelium: 850 g.

Weight of griseofulvin extracted from mycelium: 12.0 g.

#### RELATION OF CHLORIDE CONTENT OF MEDIUM TO PRODUCTION OF GRISEOFULVIN

Chlorine is not generally considered essential for the growth of fungi, but it is clearly essential for the production of griseofulvin. Allowing for the probable inefficiency of the methods of extraction used it seems likely that the real yield of griseofulvin per litre of culture (in both mycelium and filtrate) is of the order of 500 g. This would require that the fungus be supplied with 50 mg. chlorine per litre or approximately 0.01 % KCl. A concentration of KCl of this order is necessary to obtain maximum titres in cultures of *P. janczewskii* as will be seen by reference to the experimental data in Table 2. The fact that assays were obtained in previous experiments

Table 2. Relation between chloride content of medium *N* and production of griseofulvin by *Penicillium janczewskii*

Potassium chloride (%)	Assay (days)			
	4	6	8	10
—	S <sub>2</sub> C <sub>32</sub>	S <sub>16</sub> C <sub>256</sub>	S <sub>8</sub> C <sub>256</sub>	S <sub>8</sub> C <sub>256</sub>
0.001	S <sub>4</sub> C <sub>64</sub>	S <sub>32</sub> C <sub>1024</sub>	S <sub>64</sub> C <sub>2048</sub>	S <sub>128</sub> C <sub>1024</sub>
0.005	S <sub>2</sub> C <sub>64</sub>	S <sub>32</sub> C <sub>1024</sub>	S <sub>64</sub> C <sub>4096</sub>	S <sub>256</sub> C <sub>8192</sub>
0.01	S <sub>8</sub> C <sub>128</sub>	S <sub>32</sub> C <sub>1024</sub>	S <sub>128</sub> C <sub>4096</sub>	S <sub>128</sub> C <sub>8192</sub>
0.1	S <sub>2</sub> C <sub>64</sub>	S <sub>32</sub> C <sub>1024</sub>	S <sub>128</sub> C <sub>4096</sub>	S <sub>256</sub> C <sub>8192</sub>
1.0	S <sub>4</sub> C <sub>128</sub>	S <sub>64</sub> C <sub>1024</sub>	S <sub>64</sub> C <sub>4096</sub>	S <sub>128</sub> C <sub>4096</sub>

(Brian *et al.* 1946) on media with no added chloride (e.g. medium *N*) can be accounted for by impurities in the dextrose used and HCl used for adjusting the initial pH. The composition of medium *N* is as follows: potassium nitrate, 2.3 g.; potassium dihydrogen phosphate, 1.0 g.; hydrated

magnesium sulphate, 0.5 g.; dextrose, 75.0 g.; minor element concentrate, 1 ml.; distilled water, 1000 ml. In this experiment (Table 2) all constituents were of the purest grade available and pH was not adjusted but, nevertheless, there was apparently sufficient chloride present to result in the development of appreciable 'curling activity'. The highest concentration of potassium chloride (1.0 %) somewhat reduced the vigour of growth of the mould.

#### SUMMARY

The 'curling factor' produced by *Penicillium janczewskii* has been shown to be identical with the substance known as griseofulvin ( $C_{17}H_{17}O_6Cl$ ) previously isolated from the mycelium of *P. griseofulvum* and *P. patulum*. All strains of *P. janczewskii* examined, from different sources, have shown the capacity to produce griseofulvin, but not all strains of *P. griseofulvum* have done so.

Yields of griseofulvin from *P. janczewskii* can be approximately doubled by extracting the mycelium as well as the culture filtrate; by this means yields of the order of 400 mg. per litre of culture medium have been obtained. A chlorine content of the medium corresponding to 0.005–0.01 % potassium chloride is optimum for griseofulvin production.

We are much indebted to Prof. H. Raistrick, F.R.S., for the provision of authentic samples of griseofulvin and cultures of *P. griseofulvum*.

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# STUDIES ON SOME DISEASES OF SAINFOIN (*ONOBRYCHIS SATIVA*)

## II. THE LIFE HISTORY OF *RAMULARIA* *ONOBRYCHIDIS* ALLESCHER

By S. J. HUGHES

(With Plate VI and 22 Text-figures)

*Ramularia onobrychidis* Allescher causes a leaf spot of sainfoin. Attacked leaflets are killed and fall away readily from the midrib and in a thick crop the disease can cause quite a heavy loss of leaflets. In Great Britain it has been recognized only in the Vale of Glamorgan, South Wales. It was found there first in December 1943 on the local strain of common sainfoin and later also on the Hampshire and Cotswold commercial strains.

### HISTORICAL REVIEW

The fungus was first described by Allescher (1891) as follows: '*R. Onobrychidis* Allescher nov.spec. Maculis circularibus, brunneis; caespitulis hypophyllis; conidiis cylindraceis, rectis vel leniter curvatis, utrinque obtusiusculis, plerumque 1 septatis, hyalinis, ca.  $20-30 \times 3-5$ . Hab. in foliis vivis *Onobrychidis sativae* in campis prope Pasing juxta München Bavariae superioris; leg. Allescher.'

Prillieux and Delacroix (1893) used the same name to describe a *Ramularia* on sainfoin thus: '*Ramularia Onobrychidis*, nov.sp., parasite sur les feuilles de Sainfoin (*Onobrychis sativa*).

Le *Ramularia* qui accompagnait de temps en temps ces périthèces d'*Ascochyta* (*A. Orobí* var. *Onobrychidis* Prill. & Delacr.) se présente comme de très petites taches, blanches, pruineuses; ses sporophores simples, septés, d'environ  $50 \times 3 \mu$ , se terminent par une courte chaîne de conidies de  $15-30 \times 4.5-5 \mu$ , plus ou moins atténuées ou arrondies au deux extrémités, continues au début et qui acquièrent deux ou trois cloisons à la maturité.

Cette mucédinée nous paraît être une form conidienne de l'*Ascochyta* dont nous venons de parler.'

Saccardo (1895), when compiling *Ramularia onobrychidis* Allesch., treated *R. onobrychidis* Prill. & Delacr. as a synonym; he reproduced both diagnoses. Although Allescher did not mention the conidiophores, it is not to be doubted that the same species was described by Prillieux and Delacroix.

No new observations have been made on the fungus since 1893, although it has been compiled by various authors with the use of the original descriptions. The fungus has been recorded in various countries: in Denmark by Rostrup (1902), in South Bavaria (Innsbruck) by Magnus (1905), North Italy by Ferraris (1913) and in Serbia by Ranojevic (1914). *R. onobrychidis* is also compiled by Massee (1915) and Jaczewski (1917), but these are doubtful records for Great Britain and Russia respectively. Stevenson (1926) recorded *R. onobrychidis* All. on *Onobrychidis sativa* and *O. viciaefolia*

in Russia, Germany, Sweden, Yugoslavia, France and Denmark. Sainfoin is also grown in British Columbia, Bulgaria, England, Finland, Holland, Switzerland and Hungary.

#### DESCRIPTION OF SPOTS

Only a single field has so far provided an abundance of leaf spots throughout the crop, but elsewhere, uncut headlands, where the air is kept moist by tall growth of sainfoin and of grasses, have provided further heavy centres of infection.

The diseased spots are very variable (Pl. VI, fig. 1). When young they are dark brown, but as they increase in size they grow paler from the centre outwards and either produce a ring spot or a uniformly pale coloured one. They are always darker on the adaxial side than on the abaxial side of a leaflet and even when a ring spot is seen on the upper side only a fawn area will be observed below. The dark colour is due to a darkening of the cell walls of the leaflet and in part to brown substances developed or deposited in some of the cells, especially certain palisade cells which are much larger than the adjacent ones.

Sporulation is restricted to dead tissues and occurs all the year round, but is most active in late spring, summer, and early autumn. Under suitable conditions numerous sporodochia are formed and their conidial chains cover both sides of the spot with a white meal. With the approach of winter the production of fresh sporodochia is superseded by the production of sclerotia.

In old spots sclerotia form a well-marked greyish ring (Pl. VI, fig. 3) and when waterlogged the individual sclerotia can be seen even with the naked eye as a circle of minute black dots enclosing the effete remains of sporodochia and of their conidiophores.

In younger spots, which have not produced sporodochia, sclerotia are aggregated towards the centre. Under moist conditions sclerotia may develop in fallen leaflets on the ground. Infected leaflets fall readily on being disturbed, or they may dry out while still attached to the midrib. They are readily blown about in the wind and this, no doubt, helps to spread infection to other parts of the same field and perhaps to neighbouring fields.

In summer the conidiophores form a circular area on the spot, but in winter the conidiophores develop from sclerotia and not from sporodochia, and, as might be expected, they are found in a mealy ring. In spots with a central group of sclerotia the conidiophores are central as in summer spots.

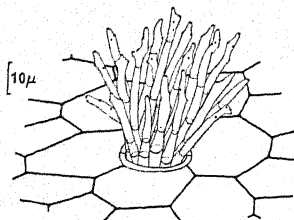
#### METHOD OF INFECTION AND DEVELOPMENT OF MYCELIUM

Examination of leaflets, inoculated by applying drops of spore suspension to their surfaces, showed that both stomatal and cuticular penetration occurs. In mounts stained with cotton blue some germ tubes were seen to pass over a stoma and pierce the cuticle some distance away, whilst others penetrated the stomatal pore. In the latter, the germ tube swelled in the substomatal cavity and then branched out into the surrounding tissue.

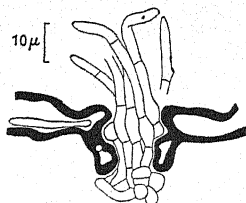
Death of the leaflet cells results in their collapse, but cells of the epidermal layers are often very readily distinguished even in old spots, presumably because of their sturdier structure. Fungal hyphae are found in all parts, but the mycelium is denser immediately beneath the epidermal layers. Hyphae are mostly intercellular, but they are also commonly found inside epidermal cells where, however, they have not been seen to develop any further. Mycelium has been seen in the phloem, but not in the xylem of the vascular bundles, whereas hyphae of *Pleospora herbarum* were encountered abundantly in both xylem and phloem elements of leaflets infected by this fungus (Hughes, 1945). The mycelium is hyaline throughout the leaflet, but as winter approaches the walls darken slightly and more so during the development of sclerotia.

#### DEVELOPMENT OF SPORODOCHIA

In *Ramularia vallisumbrosae* the sporogenous system is subcuticular (Gregory, 1939); in *R. onobrychidis* it is entirely subepidermal and substomatal. Sporodochia are restricted to the substomatal cavities and as mentioned previously, are found sporulating actively in late spring, summer and early autumn. Their tufts of conidiophores protrude through the dilated stomatal pores (Text-fig. 1) between the crushed guard cells, but in no case has any rupture of the epidermal tissues been seen.



Text-fig. 1.



Text-fig. 2.

Text-fig. 1. Sporodochial conidiophores protruding through a dilated stomatal aperture. The conidial chains have fallen away, but the scars are conspicuous. Cardiff, 11 August 1944.

Text-fig. 2. Transverse section of young sporodochium developing between and below the guard cells of the upper epidermis. Cardiff, 11 August 1944.

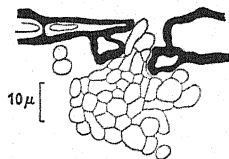
The sporodochium begins as a small knot of hyphae in the substomatal cavity, but whether this arises from one or more than one hyphal branch has not been determined. From this knot, a fascicle of hyphae, which become conidiophores, soon pushes up through the stoma; characteristic chains of spores are then developed (Text-fig. 4). The sporodochium enlarges by the division of its cells, and not uncommonly the regular outward elongation of some of the cells towards the stoma to produce conidiophores gives the sporodochium the appearance of a meristematic tissue (Text-fig. 2). Such a regular arrangement of cells in substomatal sporodochia was observed by Killian (1926) as opposed to the more irregular arrangement when sporodochia arise elsewhere. He stated: 'Les différences structurales des ébauches se manifestent encore à l'état adulte. Les conidiophores interstomataires sont formés de filaments parallèles, les autres se



présentent sous forme de pelotons globuleux.' This parallel arrangement of which he spoke is very characteristic of the 'necks' of sclerotia.

Protrusion of conidiophores through the stomata appears, according to Killian (1923, 1926) and Gregory (1939), to be less common than their rupturing of the epidermal layer.

In winter old sporodochia sometimes turn pale brown, but then seldom produce a fresh crop of spores when placed in a moist atmosphere. Killian (1926) found that sporodochia of *Ramularia parietaria* Passer. (a species which does not produce sclerotia) turned brown, especially at their bases, but retained their ability to produce conidia after a period of rest.



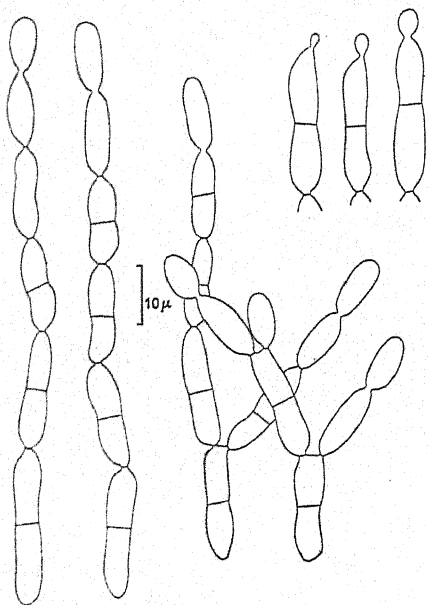
Text-fig. 3. Transverse section of young sporodochium which has not yet produced conidiophores. Upper epidermis. Cardiff, 11 August 1944.

### CONIDIOPHORES AND CONIDIA

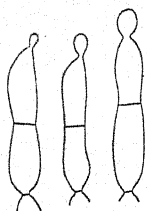
The conidiophores of the sporodochia are unbranched, filiform, hyaline, two to three or more septate with the basal cell usually swollen; they are narrower than the conidia and measure  $30-50\mu$  long and  $2-3.5\mu$  wide. Each conidial chain, after dispersal, leaves a scar on the conidiophore. As many as seven scars have been observed on a single conidiophore, but restricted to its terminal cell. The conidiophore may be geniculate or faceted, according to the position of the scar, but is usually faceted (Text-fig. 1). In young sporodochia or recently sprouted sclerotia the conidiophores bear one terminal facet, but as they become older and further spore chains develop, the position of each is clearly seen as a more refractive circle of wall substance.

The hyaline conidia vary considerably in length ( $7-42\mu$ ) and vary in form from phragmospores through didymospores to amerospores. The first conidium arises as a globose swelling at the apex of a conidiophore; it increases in length, becomes oval, and eventually almost cylindrical with a slightly tapering base. A septum separates the conidium from the conidiophore and the conidium may or may not become variously septate. The first spore is not detached from the conidiophore, but buds off another spore at its apex which becomes slightly tapering. By such successive acropetal growth a chain of dry conidia is produced. It is evident then that only the first-formed conidium is ever in actual contact with the conidiophore that bears it but: 'The first-formed spores, which are at the base of the chain, first act as part of the thallus, in that they support in the air, and carry food to, the later formed spores. "Leur séparation, qui les rend aptes à multiplier et à disperser le Champignon à la façon des spores, n'arrête pas nécessairement leur activité végétative".' (Mason, 1937.) Catenulation has been observed in all lesions and the chains may be up to seven conidia in length and quite probably longer. It is quite common to find a conidium, not necessarily the oldest, or first formed, budding off two conidia at its apex and thereby initiating a branched chain. No conidium has been seen to develop from an old scar so that the conidia are *terminus thallospores*; the production of spore chains, therefore, increases possibly tenfold the number of units presented for dissemination.

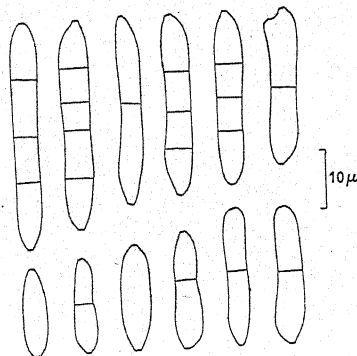
A successive reduction in the length of the conidia, as they are successively more distant from the conidiophore is evident, and in addition, the number of septa to each spore is also reduced (Text-fig. 4). Whereas the first-formed conidium is a phragmospore or didymospore, the distal conidia are invariably aseptospores; some of the longer unseptate conidia may well be potential didymospores. The production of successively smaller conidia with fewer septa is to be expected. Bliss and Fawcett (1944) have found



Text-fig. 4.



Text-fig. 5.



Text-fig. 6.

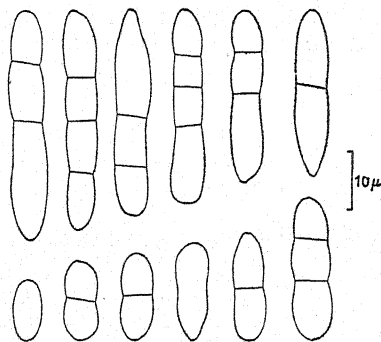
Text-fig. 4. Branched and unbranched chains of conidia deposited on a glass slide by gently tapping a fruiting lesion over it. The gradual and successive reduction of conidial length in each chain is quite evident. Southerndown, 18 March 1944.

Text-fig. 5. Development of conidia by apical budding. Gileston, 18 March 1944.

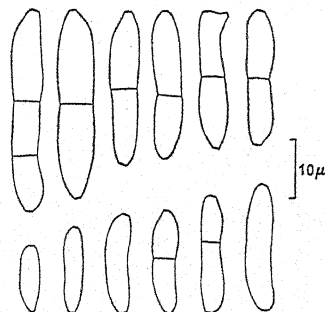
Text-fig. 6. Conidia from leafspots on sainfoin collected from Experimental Plot, Ely, Cardiff, during a very dry spell. 11 August 1944. The conidia are conspicuously narrower than those of Text-figs. 7-10.

that a similar decrease in spore length takes place in the spore chains of *Alternaria citri* Ellis & Pierce and state: 'This is perhaps due to the relative availability of food at different points along the spore chain. In this acropetal type of sporulation, the first or oldest spore grows directly from the conidiophore; but the second spore, at the apex of the first, must obtain its food by translocation through cells of the first spore. Similarly, food for succeeding spores must pass through all the older spores in the chain, a process that naturally becomes more difficult at each step.... A similar effect on spore size may also result from the branching of the spore chain. Smaller spores develop after the food stream has been divided.' This may well be the explanation for the similar phenomenon found in this *Ramularia*. When diseased leaflets with sporulating spots are tapped over a dry slide,

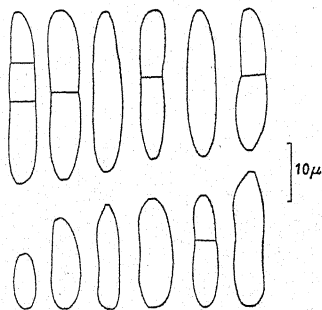
conidia or chains of conidia readily become detached from the conidiophores; by covering the slide carefully with a dry cover-slip the conidial deposit can be examined. The dissemination of conidia by an air current can be observed under a binocular microscope when a jet of air is allowed to impinge on a fruiting leaf spot. The conidia are *dry blastospores* (Mason, 1937) and in the field are presumably dispersed by wind.



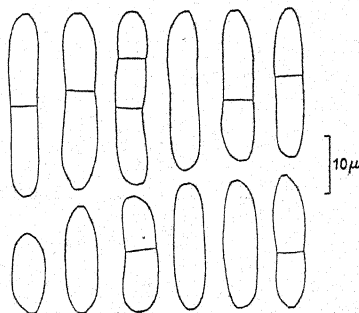
Text-fig. 7.



Text-fig. 8.



Text-fig. 9.



Text-fig. 10.

Text-fig. 7. Conidia from leafspots on sainfoin collected from dry situation. Southerndown, 18 March 1944.

Text-fig. 8. Conidia from leafspots on sainfoin collected from moist situation. Southerndown, 29 October 1944.

Text-fig. 9. Conidia from sclerotia on leaflets of sainfoin collected in fairly moist situation. Southerndown, 9 February 1945.

Text-fig. 10. Conidia from a new infection of sainfoin from fairly dry situation. Southerndown, 21 March 1945.

The spore types vary considerably in measurements and the extreme ranges from all my collections are as follows: phragmospores, 23–42  $\mu$  long; didymospores, 12–34  $\mu$  long; amerospores, 7–22  $\mu$  long.

The spores vary between 2.7 and 6.3  $\mu$  wide and the mean from all collections is 4.7  $\mu$ .

## OCCURRENCE OF THE DIFFERENT FORMS OF CONIDIA ON LEAF SPOTS

Phragmospores, didymospores and amerospores are frequently found together in the same spot, and the three spore types can be produced in the same chain. It was evident that the preponderating type of conidium was closely related to the humidity of the air at the time of conidial development. Gregory (1939) has already directed attention to the fact that *Ramularia vallisumbrosae* displays an even greater range of variation, corresponding with the form genera *Ovularia*, *Ramularia* and *Cercospora*, and he holds that 'circumstantial evidence points to humidity as one of the controlling factors'. It has already been pointed out that *Ramularia onobrychidis* is favoured by moist conditions and all but about ten of the twenty-four collections of this fungus have been made from damp situations. Again, Gregory has shown how great changes in the type of spore form, produced on a spot, are encountered when leaves are collected in tins where the atmosphere becomes moist. In my collections I took the precaution of allowing all the specimens to dry in air on trays immediately after collecting; presumably under such conditions, spore formation ceased and the spots represented more or less true field conditions.

Conidia were removed from a few spots the day after collecting, mounted in water, and fifty were measured. The type of conidium found present in preponderance was noted and the details of the collections are given in Table 1.

It is clear from Table 1 that a dry atmosphere tends to favour the production of longer conidia with more septa, whilst moist air promotes the development of shorter spores with fewer septa. There is no indication of a seasonal periodicity in the spore type encountered throughout a whole year except that in winter conditions are usually wet and the conidia, thereby, smaller as a result.

To illustrate the effect of humidity on the type of conidium formed, fruiting lesions of ten leaflets of six separate field collections were brushed lightly with a fine camel-hair brush and blown upon to remove all conidia; the leaflets were then placed in a moist chamber for forty-eight hours. Conidia were removed from the lesions at the end of this time, mounted in water and measured. Degree of septation and range in length with a mean of fifty measurements are given in Table 2. The 'field' data is that which was obtained for Table 1 from separate leaflets of the same collection.

The results of these experiments confirm the conclusion reached after a study of the data in Table 1.

Mr W. C. Moore has kindly drawn my attention (*in litt.*) to the fact that the pycnospores of his first collection of *Septoria lactucae* Pass. gave an average length of  $27.5\mu$  'but after a week of dull or wet weather most of them were appreciably longer' (Moore, 1940), with an average length of  $35\mu$ . Is it possible that such slimy pycnospores behave just the reverse to the dry blastospores of *Ramularia onobrychidis* All. and *R. vallisumbrosae* Cav., and increase in length during wet weather as do the conidia of *Cercospora* species? Another interesting observation was made by Weber (1922) on the pycnospores of *Septoria tritici*. He found that the pycnospores collected in winter were considerably larger than those collected in summer. The

summer spores measured '1.75 to 2.7  $\mu$  by 39 to 70  $\mu$ , averaging 2.2 by 50  $\mu$ ' and the winter spores measured 2.5-3.5  $\mu$  by 52-85  $\mu$  with a mean of 3 by 76  $\mu$ .

Table 1. *Summary of collections of Ramularia onobrychidis, 1943-5*

Date	Location*	Septation†	Humidity	Range in length ( $\mu$ )	Mean length ( $\mu$ )	Herb. I.M.I. no.
2. xii. 43	Ll.-M.	0 (1.2) 3	Dry	14.4-36.0	25.2	15,252
18. iii. 44	Gileston	0 (1.2) 3	Dry	15.3-36.0	24.5	—
18. iii. 44	S.down	0 (1) 2.3	Dry	10.8-36.9	25.8	—
11. viii. 44	Cardiff	0.1 (2) 3.4	Very dry	14.4-41.4	29.5	15,253
29. ix. 44	S.down	0 (1) 2.3	Moist	12.6-40.5	22.6	15,254
2. x. 44	Ll.-M.	0 (1) 2.3	"	12.6-34.2	22.4	4482
31. x. 44	S.down	(0.1) 2	"	10.8-38.7	22.0	15,255
31. x. 44	Ll.-M.	(0.1) 2	"	13.5-32.4	21.4	15,256
23. xi. 44	S.down	(0.1) 2	Very moist	12.6-32.4	20.4	15,257
23. xi. 44	Ll.-M.	(0) 1.2	"	11.7-29.7	21.0	—
12. xii. 44	S.down	(0.1)	Moist	12.6-25.2	18.9	15,258
22. xii. 44	S.down	(0.1) 2	"	10.8-33.3	21.9	15,259
22. xii. 44	Ll.-M.	(0.1)	"	10.8-27.9	19.4	15,260
5. i. 45	S.down	(0.1) 2	"	11.7-27.9	19.6	15,261
5. i. 45	Ll.-M.	(0.1) 2	"	9.9-26.1	20.1	15,262
9. ii. 45	S.down	(0.1) 2	"	10.8-33.3	20.7	15,263
9. ii. 45	Ll.-M.	(0) 1	"	14.4-25.2	19.8	15,264
22. ii. 45	S.down	(0.1) 2	Dry	9.9-36.0	22.1	15,265
22. ii. 45	Ll.-M.	0 (1) 2.3	"	12.6-39.6	19.9	15,266
7. iii. 45	S.down	0 (1) 2.3	"	11.7-37.8	22.7	—
21. iii. 45	S.down	(0.1) 2	"	10.8-36.0	20.9	15,267
25. iv. 45	Ll.-M.	(0.1) 2.3	Very dry‡	9.9-35.1	20.3	—
29. v. 45	S.down (a)	(0.1) 2.3	Moist	9.0-32.4	21.1	—
29. v. 45	S.down	0 (1) 2.3.4	Dry‡	11.7-39.6	22.5	—

\* Llantwit-Major is shortened to 'Ll.-M.' and Southerndown to 'S.down'.

† The septation of the majority of the conidia in Tables 1 and 2 is given in brackets. Thus 0 (1.2) 3 indicates that the conidia in this collection ranged between 0- and 3-septate, but were mostly 1- and 2-septate.

‡ These collections were stored in tins for a day after collecting.

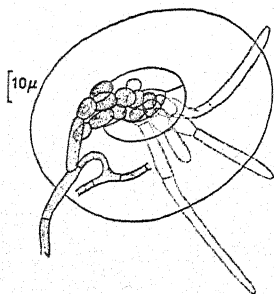
Table 2. *Effect of humidity on spore septation, range in length and mean length ( $\mu$ )*

Source	Date		Septation	Range	Mean
Gileston	18. iii. 44	(a) Field	0 (1.2) 3	15.3-36.0	24.5
		(b) Treated	(0.1) 2	11.7-29.7	20.4
Southerndown	18. iii. 44	(a) Field	0 (1) 2.3	10.8-36.9	25.8
		(b) Treated	(0.1)	7.0-28.8	17.3
Southerndown	23. xi. 44	(a) Field	(0.1) 2	12.6-32.4	20.4
		(b) Treated	(0.1)	11.7-27.9	19.1
Southerndown	22. xii. 44	(a) Field	(0.1) 2	10.8-33.3	21.9
		(b) Treated	(0) 1	10.8-28.8	17.4
Southerndown	5. i. 45	(a) Field	(0.1) 2	11.7-27.9	19.6
		(b) Treated	(0) 1	10.8-27.9	16.6
Llantwit-Major	5. i. 45	(a) Field	(0.1) 2	9.9-26.1	20.1
		(b) Treated	(0) 1	11.7-21.6	15.4

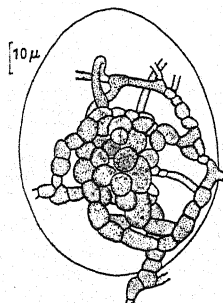
If such significant variation in spore measurement wrought by climatic conditions is common, then diagnoses must of necessity be based upon numerous collections made throughout the year.

## SCLEROTIA

Mature sclerotia consist essentially of a subglobose 'body' with a short 'neck' protruding through a much dilated stomatal aperture and slightly raised above the general level of the epidermis. The 'neck' is often markedly constricted where it passes between the guard cells. Sclerotia vary from 38 to 101  $\mu$  in diameter, and are generally produced towards the margin of a lesion (Pl. VI, fig. 3). They are formed under both upper and lower epidermis.



Text-fig. 11.



Text-fig. 12.

Text-fig. 11. Portion of strip of lower epidermis showing sclerotial initial in the substomatal cavity. The primordial cells next to the guard cells have already proliferated to produce four conidiophores which protrude through the stomatal aperture. The hyphal cells are pale brown as indicated by the stippling, whilst the outer circle denotes the lateral and well-defined limits of the substomatal cavity. Collected from a fairly moist situation. Southerndown, 5 January 1945.

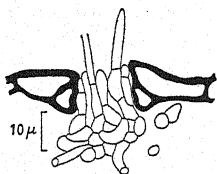
Text-fig. 12. Portion of an epidermal strip showing a young sclerotium seated in a substomatal cavity. All the cells are quite brown. Southerndown, 5 January 1945, from a fairly moist situation.

The 'body' consists of a homogeneous plectenchyma, the cell walls of which are somewhat thickened and brown, but the walls of the outermost layers of cells are only very slightly, if at all, more thickened and darker coloured than those of the core cells. The sclerotial 'neck' is characteristically composed of regularly radially arranged cells and resembles a very closely packed fascicle of pale brown and slightly thick-walled septate hyphae with rounded distal ends (Text-fig. 17). These 'neck' cells merge imperceptibly with those of the 'body'. Sclerotia were observed in small numbers in December 1943 and in abundance in January 1945, but this apparent difference is very probably due to more infection being seen in 1945 and more material being available for examination.

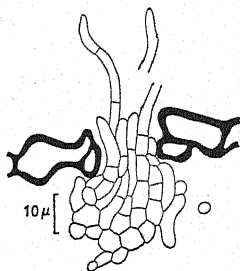
*Development of sclerotia.* The development of sclerotia has been followed from strips of epidermis of typical lesions and also from preparations of microtomed sections. A sclerotium arises from a single brown-walled hyphal branch which makes its way into a substomatal cavity. Here, it may or may not branch, but in either case proliferation occurs and a globose body is formed (Text-figs. 11–12). Further increase in size takes place by cell division and possibly by the addition of further hyphal branches on to it from surrounding mycelium. The body very soon becomes quite dark and



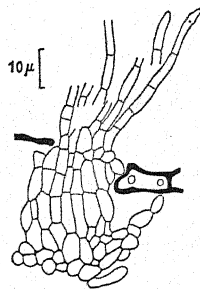
opaque and in a preparation of an epidermal strip, examined under a low magnification, the young sclerotia are seen to be seated without exception within the almost regular confines of a substomatal chamber; this is far more readily observed in strips of the lower epidermis. While the 'body' is being produced, the cells nearest the stomatal aperture become proliferated and pierce the pore, which becomes dilated as more of the 'neck' tissue is produced (Text-fig. 14). Even at this stage of development the mesophyll and palisade cells are not completely disintegrated and in particular the outlines of the mesophyll cells, with their brownish disorganized contents, are quite conspicuous. The hyphae are entirely brown-walled at this stage and staining is not necessary to follow the development of sclerotia in epidermal strips.



Text-fig. 13.



Text-fig. 14.



Text-fig. 15.

Text-fig. 13. Transverse section of a sainfoin leaflet showing stage of development of a sclerotium. Two of the visible 'neck' cells have sprouted to produce conidiophores. Collected at Llantwit-Major, 2 December 1943.

Text-fig. 14. Another stage of development of sclerotium. Llantwit-Major, 2 December 1943.

Text-fig. 15. An incompletely developed sclerotium whose 'neck' cells show considerable sprouting. Llantwit-Major, 2 December 1943.

From August 1944 until March 1945 diseased material was collected at fortnightly intervals from Llantwit-Major and Southerndown to find out when the sclerotia begin to form under the local conditions. Development began at the end of November in material from both stations, but judged by complete lack of fully formed sclerotia, very little further advance by the initials took place until late December, when mature sclerotia were seen. On leaf spots collected on 5 January 1945 and especially 22 January 1945 and later, the typical rings of sclerotia were very common. Heavy frosts and snow were experienced in December 1944 and January 1945, a fact that may well have been contributory to the development of sclerotia in such large numbers.

Sclerotial cells possess contents which stain with haematoxylin, but a deeper colour is retained by cells of the 'neck'. Crushed sclerotia liberate numerous oil globules which run together very quickly as found by Gregory (1939) in *Ramularia vallisumbrosae*, but he found that the sclerotium 'body' cells did not stain with cytoplasmic stains, although the 'neck' cells were found to be full of protoplasm. In all the sclerotia I have sectioned, no sign of any internal differentiation has been seen. Material bearing

sclerotia has been allowed to overwinter during two successive winters, one fairly mild (1943-4) and the other very severe (1944-5), but no difference in the internal structure of the sclerotia was found.

*Germination of sclerotia.* The sclerotia, whatever their true nature, germinate very readily in both field and laboratory to produce conidiophores and chains of conidia. Even sclerotial initials (Text-fig. 11) are frequently seen to sprout; in fact very few mature and fully formed sclerotia were obtained without the presence of some conidiophores coming away from the 'neck' cells. Sclerotia do not seem to require any resting period prior to germination. They do not possess a carbonaceous coat or husk as do those of *R. vallisumbrosae* and its absence may be one of the reasons for the ready germination.

The length of the conidiophores varies; in sclerotia which have sprouted naturally in the field it tends to be about the same as that of conidiophores developed in sporodochia, or often a little shorter. When sclerotia are kept under very moist conditions in the laboratory they become more elongated and may reach a length of 60  $\mu$ . Sporodochial conidiophores bear up to seven scars, but those from sclerotia have never been seen to bear more than four; and it may be that conditions were not suitable for the development of more spore chains, or adequate nutrition may have been lacking. No differences could be observed in conidia from the two types of fructifications; those from sclerotia also range from phragmospores to amerospores, depending upon the degree of humidity. The 'neck' cells are pale brown and conidiophores hyaline; the clear line of demarcation indicating the end of the 'neck' cell wall and the beginning of the conidiophore wall can frequently be observed (Text-fig. 18).

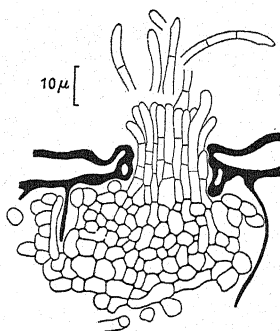
A sclerotial 'neck' similar to that found in *R. onobrychidis* has been illustrated and described by Killian (1928) for *R. sambucina* Sacc., thus: 'Ils [les sclérotés] se garnissent de prolongements filamenteux que j'interprète comme filaments conidifères, par analogie à ce que j'ai pu constater chez d'autres espèces.'

The sprouting of sclerotia to produce conidiophores and conidia is quite a common feature and has been recorded in *Sphaerella tussilaginis* Rehm (*Ramularia brunnea* Peck) by Wolf (1912), in *R. hieracii* (Bäum.) Jaap by Klebahn (1918), in *R. knautiae* (Massal.) Bubak by Laibach (1921), in *R. adoxae* Rabenh. and *R. geranii* (West.) Fuckel by Killian (1923), in *R. saxifragae* Syd. and *R. variabilis* Fuckel by Killian (1926), and in *R. vallisumbrosae* Cav. by Gregory (1939). Sprouting has also been observed in *Heterosporium gracile* Sacc. by Tisdale (1920) and in *Didymellina dianthi* Burt (*Heterosporium echinulatum* (Berk.) Cooke) by Burt (1936). There is, of course, no reason why the sclerotium should not sprout under moist conditions as it is purely vegetative tissue. In a similar manner perithecial walls have been seen to sprout in *Mycosphaerella tecomae* Wolf (*Cercospora sordida* Sacc.) by Wolf (1943).

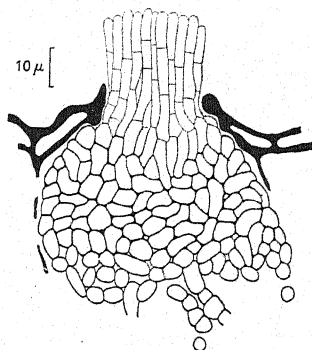
After sclerotia of *Ramularia onobrychidis* have produced a crop of conidia, and are crushed, no oil globules are liberated; presumably the oil has been used up in the production of conidia. Only one crop of spores could be obtained from sclerotia.

THE TYPE COLLECTION OF *RAMULARIA ONOBRYCHIDIS* PRILL. & DEL.

Through the kindness of Dr Arnaud and Prof. R. Heim, type material of the Prillieux and Delacroix name has been available for examination. It consists of an undated collection by G. Delacroix at Maine (Seine-et-Oise) and shows leaf spots similar to those described in this paper. No sclerotia were present and the collection is very probably a summer one. A portion of a leaf spot was teased apart and warmed in lactic acid with cotton blue. Thirty conidia gave a range of  $8-33 \times 2.5-5 \mu$  with a mean of  $19 \times 4 \mu$ ; the conidia varied between 0- and 3-septate and were mostly 1-septate (Text-fig. 19). The conidia are narrower than those observed in my collections, but this discrepancy is due to the fact that the French material was mounted dead in lactic acid and the South Wales material was mounted in water in a fresh condition. Conidia from leaf spots of a collection from Llantwit-



Text-fig. 16.



Text-fig. 17.

Text-fig. 16. An almost mature sclerotium showing parallel arrangement of 'neck' cells with rounded ends. Some sprouting has occurred. Llantwit-Major, 2 December 1943.

Text-fig. 17. A mature and unsprouted sclerotium. The 'body' is composed of a homogeneous plectenchyma with no indication of any internal differentiation. There is no definite outer layer of cells with thicker and darker walls to form a 'rind'. Llantwit-Major, 2 December 1943.

Major (2 October 1944) were mounted in lactic acid and cotton blue and thirty conidia were between  $3.5$  and  $5 \mu$  wide with a mean of  $4 \mu$ ; the fresh collection mounted in water had conidia  $3.6-6.3 \mu$  wide with a mean of  $4.7 \mu$ . The conidiophores arise from substomatal sporodochia and reach a length of  $40 \mu$  and are  $3-4 \mu$  wide; they are geniculate or faceted. There is no doubt that the French material and my own are identical.

No type material of the name *R. onobrychidis* Allescher has been available, but I do not doubt that the two names apply to the same fungus.

A published exsiccatum of *R. onobrychidis* All. is cited by Lindau (1910, p. 768), 'Exs. Vill. *Fungi Bavar.* 882'; this is from the type locality, but it has not been available for examination.

#### ISOLATIONS

Cultures of the fungus were obtained from single phragmospores, didymospores, and amerspores and only slight differences were seen in the resulting colonies. In all, twenty cultures were started from material from

different localities and collected at various times of the year, but in the following observations on the growth of the fungus on various media six isolations were used. Four of these were derived from summer infections and two from sprouting sclerotia. The media—Glucose agar, Glucose peptone agar, Czapek's agar, Oatmeal extract agar and Prune agar—were prepared as suggested by Gregory (1939). Observations were made on colonies on agar slants about six or seven weeks after inoculation and they were kept under laboratory conditions for this period.

*Glucose agar.* Colony of staling type, flat, maximum radius, 3–4 mm.; aerial mycelium very sparse, white to 'deep mouse gray'\*; immersed mycelium 'dusky olive-green'; medium not noticeably pigmented; no sclerotia; no conidia.

*Glucose peptone agar.* Colony of staling type, raised, maximum radius 15 mm.; aerial mycelium very sparse, white to 'Chaetura drab'; immersed mycelium 'dull blackish green'; medium not noticeably pigmented; no sclerotia; conidia very few, unseptate.

*Czapek's agar.* Colony of staling type, flat, maximum radius 5 mm.; aerial mycelium scanty, 'Chaetura drab'; immersed mycelium 'dull blackish green'; medium not noticeably pigmented; no sclerotia; no conidia.

*Potato dextrose agar* (1% dextrose, 2½% agar). Colony of staling type, raised, maximum radius 22 mm.; aerial mycelium abundant, white to very pale 'flesh pink'; medium 'Dresden brown' around colonies; sclerotia few, black, immersed, scattered, sometimes confluent; conidia few, unseptate and 1-septate.

*Prune agar.* Colony of staling type, raised, maximum radius 30 mm.; aerial mycelium abundant, white to very pale 'flesh pink'; submerged mycelium of colonies dark brown centrally; medium not noticeably pigmented; no sclerotia; no conidia.

*Oatmeal extract agar.* Colony of staling type, flat, maximum radius 30 mm.; aerial mycelium scanty, white; submerged mycelium of colonies dark brown centrally; medium not noticeably pigmented; few imperfect sclerotia, dark brown to black; conidia very abundant, unseptate and 1-septate, mostly unseptate ( $7.0\text{--}25.0 \times 3.5\text{--}5.5\mu$ , mean  $15.6 \times 4.6\mu$ ).

*Sainfoin agar* (sainfoin leaf and stem decoction, 2½% agar). Colony of staling type, raised, maximum radius 10 mm.; aerial mycelium abundant, white to very pale 'flesh pink'; medium too dark to observe pigmentation, but undersides of colonies dark brown centrally; no sclerotia; conidia abundant, unseptate and 1-septate.

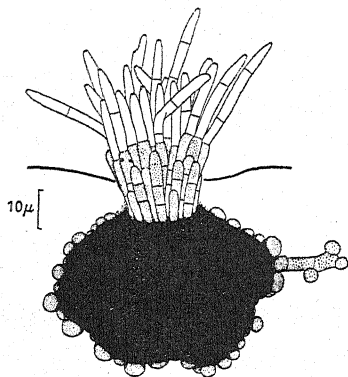
On sainfoin agar, conidiophores were produced over the raised surface of the colonies and also on the sparse surrounding mycelium, more especially during drying out or where a colony was in contact with the glass surface.

Thirty conidia gave range of  $8.5\text{--}22.5 \times 3.5\text{--}5.5\mu$  with a mean of  $14.1 \times 4.1\mu$ . The mode of production of the conidia is shown in Text-fig. 20.

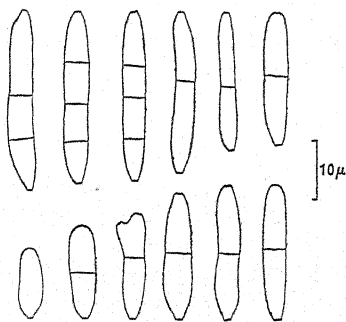
The conidiophores are short at first, but become elongate and septate; they arise at right angles from the sinuous surface hyphae and stand up

\* All colours enclosed in single quotes were obtained by reference to Ridgway's *Colour Standards and Nomenclature*.

erect on the medium although some conidiophores are very long and rest on the surface. Two or three conidial chains are produced, and branching of the individual chains frequently occurs. Complete chains of asexual spores have been seen only in culture.



Text-fig. 18.



Text-fig. 19.

Text-fig. 18. Hyaline conidiophores produced from the pale brown (stippled) 'neck' cells of a sclerotium. Collected 2 December 1943 from Llantwit-Major, allowed to dry and sprouted in a moist atmosphere nine months later and cleared in potash.

Text-fig. 19. *Ramularia onobrychidis*. Conidia from the type collection of '*R. Onobrychidis* Prillieux & Delacroix' (1893).

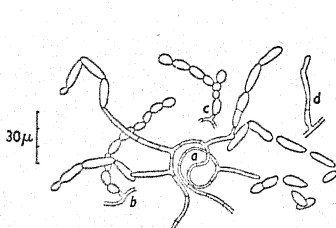
#### INOCULATION

Difficulty was encountered in infecting sainfoin leaves successfully. When potted plants were spray inoculated with a spore suspension and kept under a bell-jar, the leaflets fell away in a few days. When a spore suspension was applied to the leaflets in the form of drops from a fine pipette the drops either rolled off immediately or fell off later with the slightest shock. Brushing the leaflets with a fine camel-hair brush tended to promote the adhering of the infection drop, but the leaflet might have been damaged by this treatment; in any case the leaflets were shed. In a few cases, however, the development of lesions on leaflets of detached leaves, inoculated with drops of spore suspension in a moist chamber, was observed and sporodochia were produced. Conidia from these sporodochia were planted on agar plates and typical colonies of *R. onobrychidis* resulted.

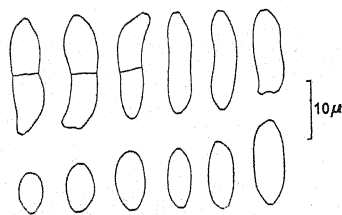
#### INTERPRETATION AND DISCUSSION

As this life-history study is based upon material collected from localities which are almost identical geographically, the interpretation of the life cycle may hold good only for this area. In Glamorgan, young leaf spots, possibly the result of recent infections, have been seen as late as November, and overwintering lesions bearing sclerotia can be seen producing conidia until February or March. It may be that conditions which permit the sprouting of sclerotia in the field also permit infection to take place. However, no new leaf spots were observed from early November 1944

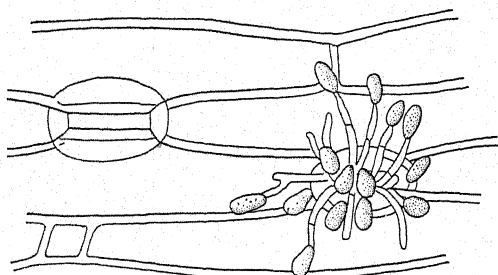
until the end of March 1945, though spores were being produced from sclerotia in the intervening period. Possibly the spores remain viable on the surfaces of the leaves, as in *R. geranii-pusilli* (Killian, 1926), but this is not very likely because the young leaves which bear the spring infections were in bud during the preceding winter.



Text-fig. 20.



Text-fig. 21.



Text-fig. 22.

Text-fig. 20. Production of conidia in culture on sainfoin agar: *a*, sinuous surface hypha bearing four long conidiophores; *b-c*, two short conidiophores bearing chains of amerspores; *d*, a geniculate conidiophore.

Text-fig. 21. Conidia from seven-weeks-old culture on sainfoin agar.

Text-fig. 22. *Ramularia pusilla*. Reproduction, by camera lucida, of the original figure of *R. pusilla* by Unger, F. (1833, Tab. II, Fig. 12).

The dry spring of 1945 undoubtedly kept the leaf spot in check in the two four-year-old fields where a plentiful supply of diseased material was obtainable previously. At the end of May, however, a crop of English common sainfoin, sown in 1944 (Southerndown (*a*) in Table 1), came to my notice. It was a thick crop, ready for the first hay cut, and notwithstanding that the two previous months had been fairly dry the lower leaves were heavily spotted by the *Ramularia*; the dense leafy cover of the crop would, of course, conserve a moist atmosphere around the bases of the plants and promote infection.

Subject to further observations a measure of control of the disease would probably be obtained by taking an earlier hay cut to prevent spotting and shedding of all the lower leaflets; consequently the plants would dry out after cutting and further loss would be checked. It is rather interesting that the leaf spot should have been so widespread, and infection so heavy, in a crop of sainfoin in its second year; it is not impossible for the disease to have been seed-borne, or rather, pod-borne.



Dried leaflets bearing sclerotia have been stored for nine months indoors, but after this storage the sclerotia were still alive and sprouted in a moist atmosphere (Text-fig. 18). Provided that sclerotia remain dry they will not sprout, but in any case in winter the low temperature probably causes conidial production to be sluggish and protracted even under suitable moist conditions.

From the work of Killian (1923, 1926, 1928) and Gregory (1939) alone, it is clear that sclerotia may be (a) perithecial initials which have not experienced and received the essential meteorological and nutritional conditions necessary for the ultimate development of asci; (b) perithecial initials which have lost their ability to produce asci; (c) initials of pycnidia producing slimy conidia as in *R. lamprosanæ*; or (d) simple vegetative aggregations of cells produced for overwintering and intended to serve in a vegetative capacity only or, in other words, overwintering sporodochia.

When their structure is considered it seems unlikely that the sclerotia of *R. onobrychidis* represent any of the first three categories. In Glamorgan it has already been shown that for two years sclerotia have given no indication that they are other than cellular aggregations on which a crop of conidiophores and conidia is borne. It is possible that in other countries or under suitable climatic conditions in this country some of the sclerotia may develop into perithecia, but no one has yet described a perfect stage.

A comparison of sporodochia and sclerotia is very interesting as it shows that both arise substomatically beneath both upper and lower epidermis. Furthermore, sclerotia, like sporodochia, are quite undifferentiated internally and are very disposed to sprout even from their first formation with the conidiophores piercing the stomatal pore as with sporodochia. Sclerotia differ from sporodochia only in their larger size, their brown-walled cells and the presence of oil globules; sporodochia do not seem to store oil in this way. These facts suggest that the sclerotia of *R. onobrychidis* are merely winter forms of sporodochia and are the means of overwintering. It is pertinent to note here that the first sporodochia found in the spring of 1945 were distinctly pale brown, but otherwise similar to the hyaline sporodochia met with later in the season.

*Slimy conidial stage.* Following Klebahn, Mason, and Gregory, an attempt has been made to determine whether there exists in *R. onobrychidis* a slimy conidial stage in the form of a pycnidium. Immature minute (?) pycnidia up to  $100\mu$  in diameter, which lacked spores, have been found in sections of leaflets infected with *Ramularia*, but these were not directly mixed with the sclerotia; it is only in leaflets bearing these overwintering bodies that they have been seen. They begin as a knot of brown-walled hyphae generally in the substomatal cavities, sometimes embedded in the disorganizing leaf tissues, but there is no indication of any terminal proliferation as with sclerotia. The core cells stain deeply with haematoxylin, indicating dense cytoplasmic contents; a cavity arises in the centre, but whether this was lysigenetic or schizogenetic could not be determined. In the most mature (?) pycnidium seen (Pl. VI, fig. 6) the original thick brown wall had been reduced to one or two layers of brown-walled cells lined by a thicker layer of deeply staining cells. From this inner lining, branching hyphal strands stretch through the cavity in all directions. A slight papilla

is formed and an ostiole develops at the apex. This structure may belong to *Ramularia* or another fungus might have been present.

A *Phyllosticta* has been observed twice on sainfoin leaflets, with conidia measuring  $4.7-7.5 \mu \times 2.0-3.0 \mu$  with a mean of  $5.5 \times 2.5 \mu$ . In culture the *Phyllosticta*, which has not been assigned to any species, in no way resembled cultures of *Ramularia* and there is no reason to assume any genetical connexion between the two. Inoculations carried out with the *Phyllosticta* were unsuccessful, and it is presumably a saprophyte.

Prillieux and Delacroix (1893) thought that their *Ascochyta orobi* var. *onobrychidis* which attacks sainfoin was genetically related to their *Ramularia*. Numerous cultures of an *Ascochyta* from sainfoin, presumably identical with that under consideration by these authors, have been made; the two fungi have often been found together on the same leaflet, but they are distinct. *Septoria orobina* Sacc., another imperfect fungus found on sainfoin, also appears to be distinct from *Ramularia onobrychidis*.

*Ascigerous stage.* Some so-called species of *Ramularia* have been shown to be merely the conidial apparatus of different species of *Sphaerella* (Fr.) Rabenh. (*Mycosphaerella* Johanson). *Sphaerella onobrychidis* Hollós has been described from sainfoin (Saccardo & Trotter, 1913), but has not been recorded in Britain and it may prove to be based on the perithecium of one of the sainfoin parasites. Old and weathered leaves and stems of sainfoin have frequently been examined and the perithecia of five different species of Pyrenomycetes have been found since 1942. Only two of these have yet been identified: (a) *Pleospora herbarum* (Pers. ex Fr.) Rabenh., which is very common on old stems and leaves, and (b) *Phomatospora berkeleyi* Sacc. (syn. *Sphaeria phomatospora* Berk.), found on old sainfoin stems, Cardiff, April 1944; this has been cultured on various media, but its colonies show no resemblance to those produced by *Ramularia onobrychidis*. A species of *Leptosphaeria* that developed on dying leaves of sainfoin in the laboratory also differs from the *Ramularia* in culture.

#### THE ORIGINAL SPECIES OF *RAMULARIA* AND A HISTORY OF THE GENUS

*Ramularia onobrychidis* Allescher can be included comfortably in the genus *Ramularia* as delimited by Saccardo; however, this author was wont to supply his own circumscription to various genera with the exclusion of the type species. For this reason it was considered worth while to trace the history of the genus and to determine the fate of the original species.

In 1833 Unger described and figured two species of *Ramularia*, *R. pusilla* and *R. didyma*, with a brief generic description in German. A translation of the German runs as follows: 'In the same way as in "*Cylindrospora*" the higher formation of the entophytic hyphomycetes proceeds from the stomata of the epidermis. In this case, however, it is no longer the simple spores but added to these is a regular conidiophore which emerges in a more or less fasciculate manner from the stomates. We name this form *Ramularia* and so far, we have had the occasion to examine two species of the genus.'

The two species were described as follows: '*R. pusilla* (Tab. II, Fig. 12), floccis erectis, subramosis, sporidiis pellucidis ovalibus, minutis, auf missfarbenen Flecken der *Poa nemoralis*' and '*R. didyma*, floccis erectis,

articulato infractis, subramosis, sporidiis ovato-cylindricis, didymis, auf Blättern von *Ranunculus polyanthemos* Lk. (Tab. II, Fig. 10, a).'

According to Wollenweber (August, 1913), Solheim (1929) and the Nomenclature Committee of the British Mycological Society (*Trans. Brit. mycol. Soc.* xxiv, p. 292, 1940) Unger did not describe the genus *Ramularia*; but this does not seem to be the case. There is a generic description and accordingly *Ramularia* may rank for priority from 1833 and not from Corda in January 1842 (*Icones fungorum* v, Prague).

Text-fig. 22 is a camera lucida reproduction of Unger's figure of *R. pusilla*. Nomenclators of the two original species are given below in chronological order.

*Ramularia pusilla* Unger, in *Die Exanthea der Pflanzen*, Vienna, p. 169, 1833.

≡ *Caeoma pusilla* (Unger) Bonorden, in *Handbuch der allgem. Myk. als Anleit.* . . . Stuttgart, p. 323, 1851.

≡ *Ovularia pusilla* (Unger) Saccardo, in *Fungi ital.* t. 970, 1881.

≡ *O. pusilla* (Unger) P. A. Saccardo & D. Saccardo, in *Sylloge fungorum*, xviii, p. 531, 1906.

≡ *O. pusilla* (Unger) [Lindau], in Rabenhorst's *Kryptogamen-Flora*, Band I, Abt. viii, p. 235, 1907.

Type collection: not specified.

Locus natalis: on discoloured spots of *Poa nemoralis* (Gramineae).

Locus classicus: not stated, presumably Germany.

*Ramularia didyma* Unger (loc. cit.).

≡ *Didymaria Unger* Corda, in *Anleit. z. Stud. der Mykologie*. Prague, p. 199, August 1842.

≡ *Puccinia didyma* [(Unger)] Bonorden (op. cit.), Taf. I, fig. 23, 1851.

≡ *P. decumbens* Bonorden (op. cit.), p. 324, 1851.

≡ *P. Unger* [(Corda)] Bonorden, in *Zur Kenntnis einiger der wichtigsten Gattungen der Coniomyceten und Cryptomyceten*. Halle, p. 53, 1860.

≡ *Didymaria didyma* (Unger) Pound, in *American Naturalist*, xxiii, p. 163, 1889.

≡ *D. didyma* (Unger) Schroeter, in *Kryptogamen-Flora von Schles.* iii, 2, p. 484, 1897.

Type collection: not specified.

Locus natalis: leaves of *Ranunculus polyanthemos*.

Locus classicus: not stated, presumably Germany.

In January 1842 Corda (op. cit. p. 7) redescribed the genus as follows: '*Ramularia* Unger Exanth. Taf. II, fig. 12 emend. Flocci entophyllini, repentes, continui, dein erumpentes. Sporae acrogenae, simplices; nucleo firmo.' Corda included only Unger's first species in the emended genus. In view of these facts *R. pusilla* should be considered the type species.

Fries (1849, *Summa Veg. Scan.* p. 493) described '*Ramularia* Ung. Fr.' as follows: 'Flocci discreti, e mycelio repente (subentophyllo) surgentes, ramosi, vulgo septati. Sporae solitariae, terminales, septatae.' Of the two original species *R. didyma* was included but not *R. pusilla*.

In 1851 (p. 41) Bonorden stated that '*Ramularia pusilla* Unger gehört ebenfalls zu dieser Gattung [*Caeoma*] wie ein Vergleich der Fig. 19 mit Fig. 42 [*Caeoma cinnamomeum*] deutlich zeigt'. Bonorden's fig. 19 is clearly

a reproduction of Unger's original of *R. pusilla* and in the 'Erklärung der Abbildungen' fig. 19 is described as '*Caeoma (Ramularia U.) pusillum...*' This is a validly published new combination which may be cited as *Caeoma pusillum* (Unger) Bonorden. In 1860 (p. 11) Bonorden again takes up *C. pusillum* and cites *Ramularia pusilla* Unger as a synonym. He saw no grounds for allowing the genus *Ramularia* to stand as one (of the original) species had simple spores and the other two-celled spores, the latter having been taken up by Corda in 1842 as the original species of another genus. Furthermore he stated that *R. pusilla* stood in complete agreement with *Caeoma* in all essential characteristics.

In 1865, in *Fungi europaei* No. 874, Rabenhorst distributed a fungus on leaves of *Alchemilla vulgaris* under the name of *Ramularia pusilla* Unger. This is the first published exsiccatum called *R. pusilla*.

After 1833 numerous species of *Ramularia* were described with conidia indicated as being septate or non-septate. In 1880 (*Michelia*, II, p. 20) Saccardo redescribed *Ramularia* Unger and cited *R. urticae* Ces. and *R. cynarae* Sacc. as examples. Later, in 1886 (*Syll.* IV, p. 196), he claimed that he had emended the genus. Also in 1880 (op. cit. p. 17) Saccardo started off the genus *Ovularia* and then in 1881 (*Fungi ital.* t. 970), he made the new combination *O. pusilla* (Ung.) Sacc., and like Rabenhorst before him in 1865, he described the fungus as occurring on leaves of *Alchemilla vulgaris* and not *Poa nemoralis* the type host as given by Unger.

Spegazzini (1879 in *Decad. mycol. Ital.* No. 105) had already described *Ramularia aplospora* on *Alchemilla vulgaris* and this was cited as a synonym of *Ovularia pusilla* by Saccardo in 1882 (*Michelia*, II, p. 547).

Magnus (1904) drew attention to the confusion and made the new combination *O. aplospora* (Speg.) Magnus for the fungus on *Alchemilla vulgaris*. Then, in 1906, P. A. Saccardo and D. Saccardo (*Syll.* XVIII, p. 532) pointed out that the fungus bearing the name '*Ovularia haplospora* (Speg.) P. Magn.' was the same as that on *Alchemilla* described by Saccardo (1886, *Syll.* IV, p. 140) as *Ovularia pusilla* and also identical with that issued by Rabenhorst (1865) as *Ramularia pusilla* in *Fungi europaei* No. 874 also on *Alchemilla*. P. A. Saccardo and D. Saccardo (*Syll.* XVIII, p. 531) repropoed *Ovularia pusilla* (Ung.) P. A. Sacc. & D. Sacc. for the fungus on *Poa nemoralis*, presumably intending this to replace *O. pusilla* (Ung.) Sacc. (1881, *Fungi ital.* t. 970). But this new proposal is quite unnecessary because *O. pusilla* (Ung.) Sacc. is a validly published alternative binomial for *Ramularia pusilla* Unger. The fact that, at its origin, it included an admitted misidentification does not matter—an "incidental" transference has been made' (Ramsbottom (1934) has discussed a somewhat similar nomenclatural problem).

Lindau (1907, p. 235) was also aware that Saccardo had the wrong fungus and unnecessarily repropoed the combination *Ovularia pusilla* (Ung.) [Lindau].

An examination of specimens in the *O. pusilla* folder in Herb. R.B.G. Kew disclosed that four of the five collections are on *Alchemilla vulgaris*. Two of these were examined and are, presumably, representative of *Ramularia aplospora* Speg. The fifth collection is on *Festuca gigantea*, not *Poa nemoralis* the type host.

Unfortunately Unger gave no measurements for his *Ramularia pusilla*, but in any event the key to the identity of this species, and the typification of the genus, lies entirely with the collecting of such a fungus on the type host.

Fresenius (1863) and P. A. Saccardo & D. Saccardo (1906, *Syll.* xviii, p. 531) have drawn attention to the fact that *Ramularia pulchella* Cesati described on *Dactylis glomerata* is reminiscent of, and closely related to, *R. pusilla*. Certainly *R. pusilla* should be re-collected and re-studied because, as the Rules stand at present, the type species must not be excluded from the genus however much the latter is expanded, restricted, or emended.

The second of the two original species, *Ramularia didyma* Unger, has also been the subject of a number of new combinations. In January 1842 (op. cit. p. 7) Corda excluded *R. didyma* from '*Ramularia* Unger emend. Corda'; he took up *R. didyma* as the type species of his genus *Didymaria* and he called it *D. ungeri* in August 1842 (op. cit. p. 199). In 1851 Bonorden copied Unger's figure of *Ramularia didyma*, but labelled it '*Puccinia didyma*' (Taf. I, fig. 23). In the 'Erklärung der Abbildungen' (p. 324), however, he proposed a nom.nov. for it as follows: '23 *Puccinia* (*Ramularia didyma*) *decumbens*, aus einer Spaltöffnung hervorbrechend.' In 1860 (op. cit. p. 53) Bonorden made the following citation: '*Puccinia Unger* syn. *Ramularia didyma* Unger. *Didymaria* Corda.' As far as I am aware neither *Puccinia decumbens* Bonorden, *P. didyma* (Unger) Bonorden nor *P. ungeri* [(Corda)] Bonorden has been used in subsequent literature.

Pound (1889), realizing that *Didymaria ungeri* Corda is an invalid name, made the new combination *D. didyma* (Unger) Pound. Unaware that this had already been done, Schroeter (1897, op. cit.) made the same combination thus: *D. didyma* (Ung.) Schroeter.

'*Cylindrospora*' is an orthographic variant of *Cylindrosporium* Grev. and was first employed by Unger (1833). It was used by Schroeter (1897, op. cit.) to replace *Ramularia* and he made new combinations to this genus.

In the *International Rules of Botanical Nomenclature*, Jena, 1935, p. 125, '*Ramularia* Fres. Beitr. (1863) 88, Sacc. Mich. II (1880) 20, non Ung. Exanth. (1833) 169.—T.: *R. lactea* [Desm. Ann. Sc. Nat. 3. sér. Bot. XIV (1850), 109, sub Fusisporio] Sacc. Mich. II. 549' is proposed for conservation and '*Cylindrospora* Schröt. Pilz. Schles. II (1897), 485, ex Grev.' is proposed for rejection.

Returning to the original references cited above we find that there is neither a '*Ramularia* Fres.' nor a '*Cylindrospora* Schröt.' to be found; these are miscitations.

This matter has been the subject of a contribution of the British Mycological Society Nomenclature Committee (*Trans. Brit. mycol. Soc.* xxiv, p. 292, 1940). The committee held that 'The reason for suggesting *Ramularia lactea* as the type is obscure'. Saccardo compiled this species, but made no mention of septation of the conidia, whilst Bommer & Rousseau (1884) made the combination '*Ovularia lactea* Sacc.' which is perhaps best cited as *O. lactea* [(Desm.)] Sacc. ex Bommer & Rousseau. In 1893 (p. 321) Masee made the combination '*Ovularia lactea* [(Desm.)] Mass.' Lindau (1907, p. 468) described the conidia of *Ramularia lactea* as usually non-septate.

The committee then stated: 'As Saccardo's conception of *Ramularia* has been generally accepted it would be convenient to attribute the "emended genus" to him.' The citation '*Ramularia* Sacc. *Michelia*, II, 20, (1880)' cannot be verified. Following Clements and Shear (1931) it was recommended that *R. urticae* Ces. (which was claimed to have pluriseptate conidia) be accepted as type species and not *R. lactea*; the reason given is that Cesati 'appears to have been the first to use *Ramularia* in the sense of Saccardo'. The committee accepted the exclusion of the two original species of *Ramularia* from the genus; on p. 293, however, they stated: 'In 1897 Schroeter (loc. cit.) adopted "*Cylindrospora* Grev." to replace *Ramularia* in the sense of Saccardo', and they held the view that 'As this excluded Greville's type species [*Cylindrosporium concentricum*], Schroeter's use is now invalid....'

Eleven exsiccati of *Ramularia urticae* Ces. are available and have been examined, as well as a fresh collection on *Urtica dioica* (April 1946 in Herb. I.M.I. no. 4900). In all specimens the preponderance of conidia are amero-spores with only a few didymospores. No pluriseptate conidia were observed.

Saccardo's emended diagnosis of the genus *Ramularia* Unger runs as follows: 'Biophila. Hyphae breve vage ramulosae; conidia ovato cylindracea, varia, denique 2- pluriseptata (et interdum catenulata)—Ex. *R. Urticae* Ces. & *R. Cynarae* Sacc.' (*Mich.* II, p. 20, 1880.)

What then is included in the genus *Ramularia* Unger in Saccardo's *Sylloge fungorum*? Of over 440 species and varieties compiled, in 369 of the descriptions the septation of the conidia is clearly stated; in Table 3 these are separated into eight categories depending upon the septation.

Table 3

Conidia continuous	64 species or varieties
" up to 1-septate	185 " "
" " 2- "	42 " "
" " 3- "	63 " "
" " 4- "	9 " "
" " 5- "	4 " "
" " 6- "	1 " "
" " 8- "	1 " "

Saccardo did not, therefore, restrict the genus *Ramularia* to species with conidia 'denique 2- pluriseptata'; in fact, the species with continuous or with 0- to 1-septate conidia outnumber those with conidia 2- to pluriseptate. *R. lactea* and *R. urticae* find places with the majority of species.

In 1880 (*Mich.* II, p. 17) Saccardo described *Ovularia* as follows: 'Biophila; hyphae subsimplices, erectae, apicem versus conidia globosa v. ovoidea gerentes. Est *Ramulariae* analoga—Ex. *O. obovata* Fuckel, *O. sphaeroidea* Sacc., sub *Ramularia*.' In 1886 (*Syll.* IV, p. 140), however, he added that the conidia were 'rarius breve catenulata'. Besides species with globose or ovoid conidia, Saccardo compiled in *Ovularia* fungi whose conidia were described variously as lanceolate, oblong and even cylindrical or cylindrical-ellipsoid and rarely 1-septate; in addition, the conidia of some species are described as being catenulate. The genus *Ovularia*, therefore, is not homogenous and considering the descriptions applied to conidial contour there seems to be a gradation embracing species in both *Ramularia* and *Ovularia*. This is borne



out by a consideration of the spore lengths in relation to breadths where these are given in the diagnoses.

Ellis and Everhart (1885) did not accept the genus and included all species of *Ovularia* in *Ramularia*. Pound and Clements (1896) were also inclined to consider it better to unite *Ovularia* and *Didymaria* with *Ramularia*.

Massee (1893) compiled the British species of *Ramularia* and *Ovularia*. He restricted *Ramularia* to those species with conidia '1- to many septate' and *Ovularia* to those with one-celled conidia, whilst some species in both genera were described with conidia in chains. British species appearing in Saccardo's *Sylloge* under *Ramularia* and described with non-septate conidia were transferred to *Ovularia* by Massee; *Ramularia lapsanae*, however, appears as '*Ramularia lapsanae*' (p. 345) and also as '*Ovularia lapsanae* Mass.' (p. 320).

Gregory (1939) pointed out that 'on its host plant *Ramularia Vallisumbrosae* certainly has the habit of one of the Tuberculariaceae'; the same is true of *R. onobrychidis* and of many other species of *Ramularia*. However, in Saccardo's classification *Ramularia* is placed amongst the Mucedineae. As in *R. vallisumbrosae*, the plechtenchymatous masses bearing conidiophores in *R. onobrychidis* 'must be regarded as fundamental in the economy of the fungus'. (Gregory discusses this matter in detail.) In *R. vallisumbrosae* and *R. onobrychidis* alike, dark hyphae and darker stromata occur; in Saccardo's classification, however, only the hyaline aerial conidiophores and conidia are considered.

The so-called species of *Ramularia* are generally held to be the conidial apparatus of species of the genus *Mycosphaerella*, but a *Ramularia*-like conidial apparatus is also known to be produced by some species of the genus *Entyloma* of the Ustilaginales. Marchal and Sternon (1924) described and figured how *E. oenotherae* produced tufts of short, hyaline and unbranched conidiophores. These emerged through the stomata and each conidiophore bore, apically and subapically, chains of septate and continuous conidia—evidently a dry conidial apparatus no different from what is generally assigned to *Ramularia*. The presence of chlamydospores which germinated to produce 'basidiospores' showed the fungus to be an *Entyloma*. Of eleven species of *Ramularia* which these authors investigated, chlamydospores were found in *R. armoraciae* Fuckel and *R. variabilis* Fuckel, but they were not observed to germinate. Höhnelt (1924) erected the genus *Entylomella* for the conidia of species of *Entyloma* and *Doassansia*. He claimed that the genus *Entylomella* is synonymous *pro parte* with '*Cylindrosporium* Sacc. (non Greville)'; this is an invalid citation, as *Cylindrosporium* is not Saccardo's genus—*Cylindrosporium* was proposed by Greville, and Saccardo (1880, op. cit. p. 12) gave it his own circumscription and excluded the type species; however, Höhnelt made seven new combinations to his *Entylomella*, all of which are really nomenclaturally superfluous.

Ciferri (1928) emended *Entylomella* and cited as synonyms '*Cylindrosporium* Sacc. p.p. (non Grev.)' and '*Ramularia* Fuckel, *Symb. Myc.* p. 361, 1869'. Fuckel, in this reference, listed and described species under *Ramularia* Unger not *Ramularia* Fuckel. Ciferri made three new combinations to *Entylomella* using (a) '*Ramularia oenotherae* Marchal & Sternon', (b) *R. armoraciae* and (c) *R. variabilis*. I find no trace of '*R. oenotherae* Marchal &



Sternon' in the literature, but in 1924 these authors did suggest that the conidial form they found produced by *Entyloma oenotherae* Marchal & Sternon might be identical with the fungus by the name of '*Ramularia oenotherae* Ivanoff'.

Wolf (1912) stated that the production of 0- to 3-septate conidia in *R. brunnea* in chains is 'characteristic of the genus *Septocylindrium* while in *Ramularia* the conidia are not in chains.'

Wollenweber (February 1913) cited '*Septocylindrium* Bonorden' as a synonym of *Ramularia* Unger. He stated that '*Ramularia* is a genus related to *Fusarium* but with conidia of cylindrical shape. Chlamydospores are present, but the ascigerous stage is unknown. It should not be confused with the ascomycete *Mycosphaerella*, which has similar conidia but no true chlamydospores. *Ramularia* contains wound parasites causing root troubles, stem anthracnoses, leaf spot diseases and fruit rots.' Wollenweber made the new combination '*Ramularia didymum*' (Harting) Woll. (syn. *Fusisporium didymum* Harting, *Fusarium didymum* (Harting) Lindau). The significant point of this new combination is that '*Fusisporium didymum* Harting' is a slimy spored fungus whereas in *Ramularia* the conidial chains are generally regarded as representing dry terminus thallospores which are produced in acropetal succession. In August 1913, Wollenweber published his own circumscription of the genus '*Ramularia* (Unger) Fries' so that it included fungi with chlamydospores, and conidia produced several in sequence and occasionally adhering in short chains at the end of a conidiophore. Such conidia are slimy and are produced in basipetal succession from the end of a phialide. In the same paper Wollenweber erected the new genus *Cylindrocarpon* to include fungi similar to those included by him in *Ramularia*, but which differed in the absence of chlamydospores. Later, Wollenweber transferred to *Cylindrocarpon* some of the chlamydospore-producing forms which originally he had included in *Ramularia*.

It is interesting to note that Bubák (1916) made the combination *Ramularia septata* (Bon.) Bubák (syn. *Cylindrium septatum* Bon., *Septocylindrium Bonordenii* Sacc., *S. septatum* (Bon.) Pound, *S. septatum* (Bon.) [Lindau]). Bubák stated that this fungus differs from *Cercospora* because of the catenate conidia, and from *Ramularia* because the conidia were up to 4-septate. He considered that it was impossible to erect a new genus [*Septocylindrium*] on these grounds.

Overholts (1940) held a view similar to that of Wolf when he said 'I have seen some evidence that this fungus [*Ramularia oxalidis* Farlow] would be better referred to *Septocylindrium*, since in a few cases the spores were seen in chains of two'.

Of the facts arising as a result of tracing the history of the genus *Ramularia* the most significant is that our knowledge of the type species amounts to very little; in fact but few of the 400 odd species of *Ramularia* in Saccardo's *Sylloge fungorum* have been studied in any detail to determine the seasonal variation. Until the full range of the variation displayed by *R. pusilla* is known, the generic name *Ramularia* cannot be consistently applied.

It appears that the so-called species of *Ramularia*, *Didymaria* and *Ovularia* produce a fascicle of hyaline conidiophores emerging through the leaf or stem epidermis or through a stomate, and in the few species known in

detail these conidiophores arise from an internal stroma; single conidia or chains of conidia, hyaline, variously septate or non-septate, varying in shape from spherical to ovate, obovate or cylindrical, are borne on hyaline conidiophores. As far as is known at present it is doubtful whether these three generic names can be used exclusively.

#### SUMMARY

*Ramularia onobrychidis* causes a leaf spot of sainfoin in Glamorgan, Wales.

In field material, chains of conidia are produced on conidiophores of substomatal sporodochia from late spring to early autumn. Conidia vary from phragmospores through didymospores to amerospores; moist conditions promote the formation of shorter conidia with fewer septa, and very dry conditions some longer, somewhat narrower conidia with up to four septa.

Sclerotia develop in winter in the substomatal cavities and are arranged usually in a ring around an old spot. They germinate very readily in the field under moist conditions without any resting period and retain their viability for at least nine months indoors, when kept dry. Sclerotia consist essentially of a more or less globose 'body' of homogeneous plectenchymatic cells with brown and slightly thickened walls, and a 'neck' of regularly columnar cells. It is from the apices of these neck cells that conidiophores arise. Sclerotia store oil, which disappears during the production of a single crop of conidia.

Single spore isolations of the different spore forms from summer spots and from germinating sclerotia have given similar cultures. The disease was reproduced with difficulty when healthy sainfoin leaflets were inoculated with spore suspensions and *R. onobrychidis* was re-isolated from the spots.

No perithecial stage in the life history has been discovered and it is probable that the fungus overwinters, in South Wales, by means of its sclerotia. A (?) pycnidial form, found near spots caused by *Ramularia* may represent the slimy conidial apparatus.

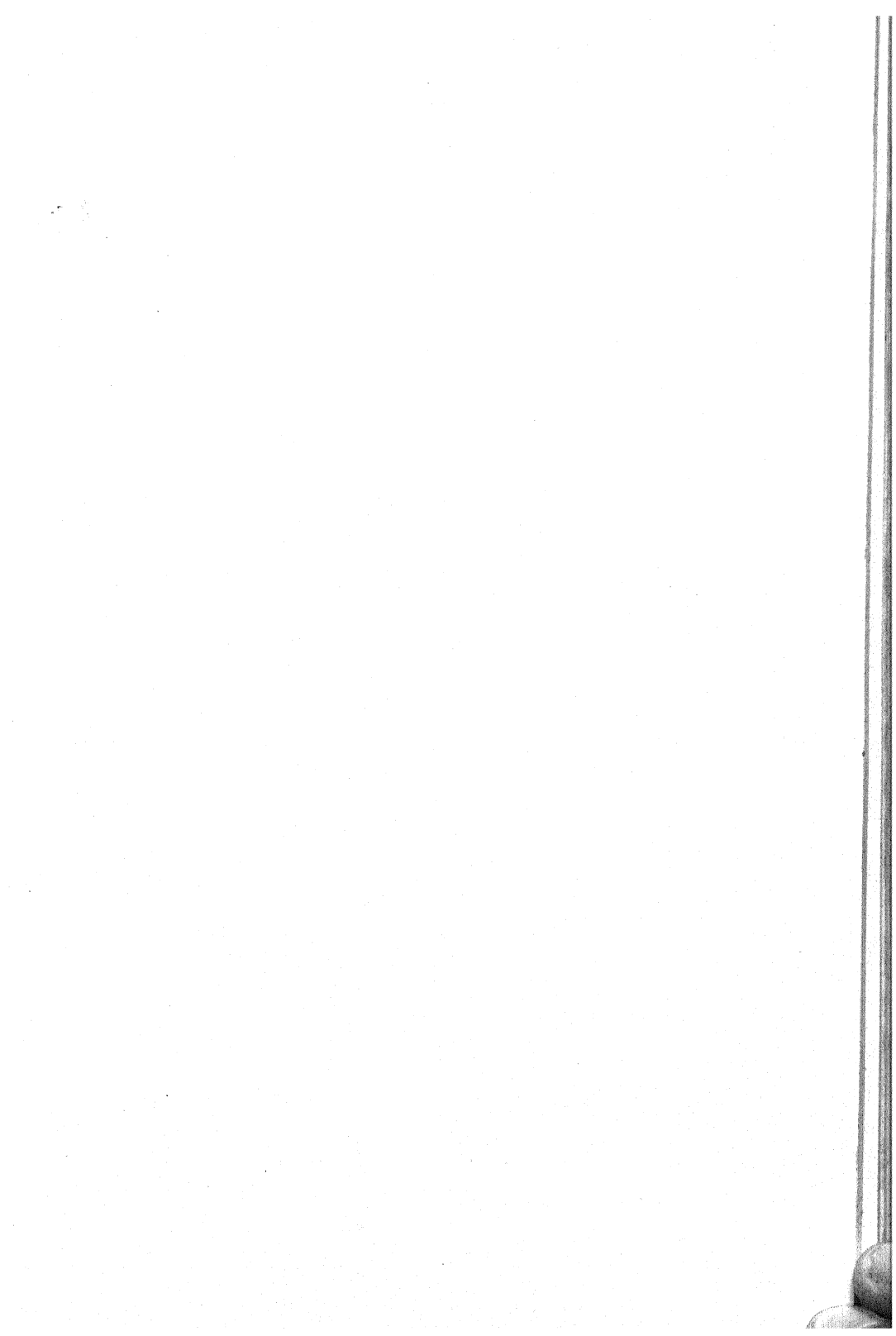
The fate of the original species of *Ramularia* is discussed and a history of the genus is given.

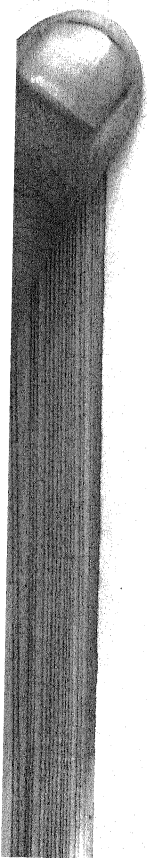
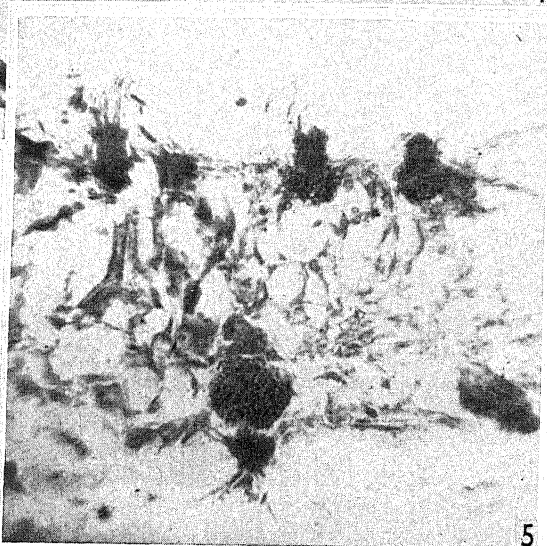
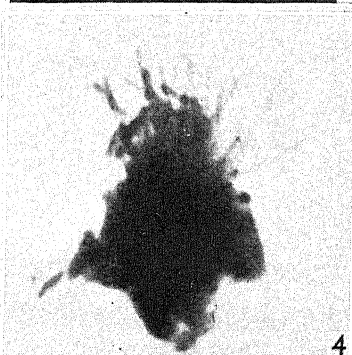
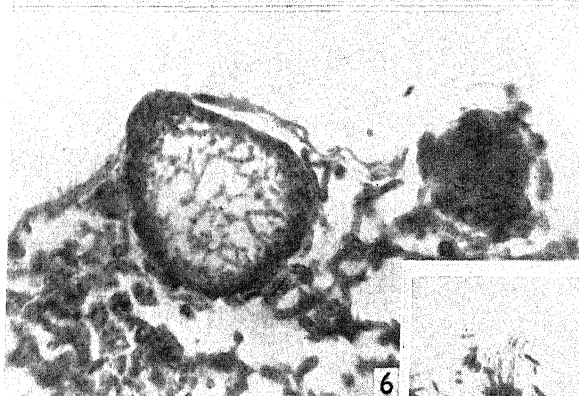
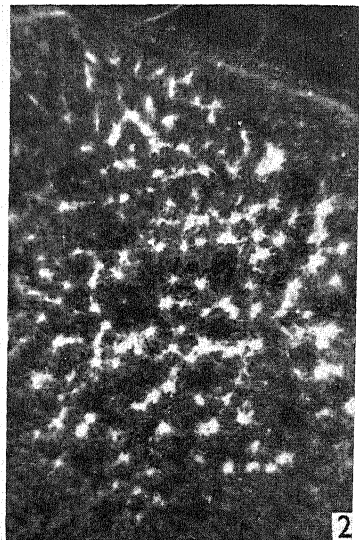
This investigation was carried out at the Advisory and Research Department in Agricultural Botany, University College, Cardiff, under the direction of Mr J. Rees to whom I am grateful for much advice. I am also indebted to Mr E. W. Mason and Mr W. C. Moore for helpful criticism. The photographs were taken by Miss K. L. Pears.

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EXPLANATION OF PLATE VI

- Fig. 1. Sainfoin leaflets showing spots. Natural size.  
 Fig. 2. Surface view of sainfoin leaflets bearing conidiophores and chains of conidia.  
 Fig. 3. Portion of sainfoin leaflet, cleared in caustic potash, showing rings of sclerotia. Collected at Southerndown, January 1945. Unstained.  $\times 7$ .  
 Fig. 4. Sprouting sclerotium teased from leaflet. Stained lightly with cotton blue.  $\times 333$ .  
 Fig. 5. Portion of transverse section of leaf spot showing sprouting sclerotia. Collected at Cardiff, December 1943, and stained in haematoxylin.  $\times 200$ .  
 Fig. 6. Young (?) pycnidium and (?) pycnidial initial from infected leaflet collected at Cardiff, December 1943. The branching strands can be seen stretching across the cavity. Stained in haematoxylin.  $\times 333$ .

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POSTSCRIPT. Since this article went to press I have found that in 1836 Unger (*Ueber den Einfluss des Bodens...*, p. 224, Vienna) wrote as follows:

'144. *Ramularia pusilla* m. Char. gen.—Floccis erectis, subramosis stomatibus plantarum innatis, sporidiis simplicibus aut septatis.—Char. spec. Floccis erectis, subramosis, sporidiis pellucidis ovalibus minutis.—Auf missfarbnen Flecken der Blätter von *Poa nemoralis* im Spätsommer.—Conf. *Exanth. der Pflanzen*. Tab. II, fig. 12.

'145. *Ramularia didyma* m.—....'

In effect Unger here selected *Ramularia pusilla* as his lectotype for the generic name *Ramularia* Unger.

I notice, too, that Sprague (*Mycologia*, XL, 3, p. 309, 1948) has identified *Ovularia pulchella* (Ces.) Sacc. with '*Ovularia pusilla* (Ung.) Sacc. and D. Sacc.' and this species is recorded on *Bromus inermis* and *B. carinatus* in U.S.A.

## STUDIES ON SOME DISEASES OF SAINFOIN (*ONOBRYCHIS SATIVA*)

### III. LEAF-SPOT CAUSED BY *SEPTORIA OROBINA* SACC.

By S. J. HUGHES

(With 1 Text-figure)

A leaf spot of sainfoin caused by a species of *Septoria* was observed in June 1942 at the Cardiff University College Agricultural Experimental Plot at Ely. The disease was neither extensive nor, apparently, causing serious damage. Some pods of the few diseased plants bore mature pycnidia of the fungus. No further attacks by this fungus have been seen in any crops of sainfoin in Glamorgan. Fortunately, the fungus was obtained in culture at its first and only encounter and this permitted a study of its cultural characters and pathogenicity. The diseased spots were more or less round, up to 6 mm. in diameter, fawn and without a distinct margin. Pycnidia showed up as minute black dots on the spot; they were scattered but generally denser at the centre.

In culture on potato dextrose agar the *Septoria* produced a conspicuous cushion (or 'heaped-up' growth). The surface of the colony was much folded, black, with some white aerial mycelium. It was extremely slow growing and a colony seldom exceeded a diameter of 1.5 cm. even after some weeks at room temperature. The fungal cushion of closely interwoven, dark-walled hyphae was cheesy in consistency, and at the surface, pycnidia of irregular shape were produced in large numbers. After subculturing many times the fungus grew more readily on various media and covered, finally, a whole four-inch plate. The colony was then hyaline towards the margin, dark towards the centre and showed slight wrinkling of the surface. The potato dextrose agar in slants was coloured a very light 'pale Vinaceous-Lilac' (Ridgway), whilst the sediment in the medium sometimes became 'Vinaceous-Lilac'. Abundant pycnidia appeared as minute raised points on the surface and pycnospores developed freely and were exuded through the pores. Pycnospores were still viable after a year at room temperature in a dried culture.

No species of *Septoria* has previously been recorded on sainfoin and the following description, based upon the original diseased material, is provided.

Pycnidia amphigenous, mostly under the adaxial epidermis, denser at the centre of the spot, subepidermal, generally in the substomatal cavity, globose to pyriform, 48–128  $\mu$  in diameter, average 73  $\mu$  and mostly 68  $\mu$ , pale brown with darker-walled cells around an irregularly circular pore. Wall membranaceous composed of two to three layers of thick-walled cells. Pore 11–23  $\mu$  wide, average 15  $\mu$ , often borne on a short papilla, opening at the surface of the leaflet. Pycnospores hyaline, filiform, straight



or sometimes slightly curved, ends rounded  $15-40\mu$  long, average  $27\mu$ , mostly  $28\mu$ , about  $0.8-1.0\mu$  wide, at first continuous and finely guttulate, later mostly 3-septate and distinctly so.

On living leaves and pods of *Onobrychis sativa* causing roundish, fawn spots with indistinct margins, up to 6 mm. in diameter. Cardiff, June 1942.

Measurements on 200 pycnospores made at intervals gave the following results:

Substrate and date	Range in length ( $\mu$ )	Mean length ( $\mu$ )	Length of most pycnospores ( $\mu$ )
Sainfoin leaves, 1942	17-40	28	28
Sainfoin pods, 1942	15-35	26	28
Potato dextrose agar, 1943	18-33	26	27
Potato dextrose agar, 1943	22-37	30	30
Potato dextrose agar, 1944	19-40	28	28

The only *Septoria* recorded on species of the genus *Onobrychis* is *Septoria onobrychidis* N. Ranoïévich, on *Onobrychis saxatilis*. According to the description, as compiled by Trotter (1931), the pycnospores measured  $28-51 \times 2.5-3.5\mu$ ; these measurements are far too large for the sainfoin fungus so the species need not, therefore, be considered further. The traditional course under such circumstances in the Coelomycetes is to describe the fungus as new. Of the species of *Septoria* recorded on herbaceous, papillionaceous hosts, one stands out, from its description, as being very similar to the sainfoin fungus. This species is *S. orobina* Saccardo (1878) and was described as follows: 'Maculis irregularibus arecendo subochraceis, atrocinctis; peritheciis remotis, punctiformibus, lenticularibus poro pertusis; spermatiis filiformibus, flexuosis,  $30 \times 0.75$ , eguttulatis, hyalinis.

Hab. in foliis Orobi verni in sylva Montello, Sept. 1874.'

No material of Saccardo's species has been available for examination, but his pycnospore measurements are so close to the mean I have obtained repeatedly for the *Septoria* on sainfoin that for the time being I prefer to call the sainfoin fungus *S. orobina* Sacc.

In culture the pycnidium of *S. orobina* Sacc. is symphyogenous in development; this can be observed in a culture of the fungus on dilute potato dextrose agar. Hyphae which are not morphologically different from others coil up irregularly to form a primordium. The mature pycnidium is spherical or pyriform with a pore situated on a papilla which may be short or long, depending, apparently, upon its depth of immersion in the medium. Two or three neighbouring pycnidia often develop in such close contact that their cavities merge into one, but separate pores are formed. The brown wall is complete all round, apart from the apical pore, and is composed of one to three dark-walled cell layers lined by thinner walled and hyaline sporogenous cells.

Pycnospores are also produced, in culture, on the individual hyphae of the mycelium (Fig. 1). They usually develop from a short lateral branch of a hypha. Groups of thirty or more spores were seen around a few short lateral branches, indicating that more than one pycnospore must have developed from each. The pycnospores are 3- to 7-septate, mostly 3-septate, but longer than those developed inside a pycnidium. The extra-pycnidial

production of pycnospores in the genus *Septoria* has previously been observed and figured by Weber (1923), and it is not an uncommon occurrence when other Coelomycetes are grown in culture.

According to Weber (1923) some species of *Septoria* which he studied produced conidia from scant hyaline mycelium, whereas in other species no extra-pycnidial conidia were produced on the abundant mycelium.

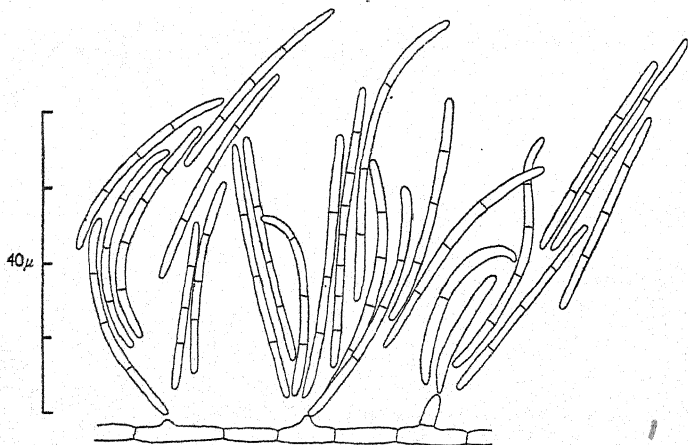


Fig. 1. *Septoria orobina* Sacc. Production of spores from a hypha in a culture on a diluted potato-dextrose-agar medium.

When sainfoin plants were spray-inoculated with a spore suspension, kept in a slightly moist atmosphere of a bell-jar for two days and then put outdoors, infection took place readily; most infected leaflets fell away after a few days. Pycnidia developed and spores were produced in ten to fourteen days. Microscopic examination showed that the germ tubes had entered through the stomata. Inoculations by spore suspensions were also made on damaged and undamaged leaves of white clover, red clover and *Vicia cracca* but no infection resulted. The fungus was still strongly pathogenic to sainfoin after two years in culture.

This investigation was carried out at the Advisory and Research Department in Agricultural Botany, University College, Cardiff, under the direction of Mr J. Rees. Preparations of the fungus have been deposited in the Herbarium of the Imperial Mycological Institute as No. 15,293.

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# THE PERITHECIA AND PYCNIDIA OF *LEPTOSPHERIA NIGRANS*

By S. J. HUGHES

(With 2 Text-figures)

W. C. Moore (1943) stated that species of *Leptosphaeria* on cereals deserve further study and 'various names have been given to them, not all of which can be relied upon'.

In April 1944, at Colwinston, Glamorgan, *L. nigrans* was found in association with an *Ophiobolus* on cereal stubble. Perithecia of the *Leptosphaeria* were sunken between the veins of the darkened sheathing leaf base and bore numerous radiating brown hyphae  $2-3.5\mu$  wide. Perithecia measured  $180-330\mu$  in diameter when viewed from above, with a mean of  $223\mu$  (twenty-three perithecia measured). The body of the depressed perithecium was dark brown and the short neck was black.

The more or less clavate asci were  $74-109\mu$  long with a mean of  $89\mu$  (eighty asci measured) and were  $9-12.6\mu$  wide with a mean of  $10.6\mu$  (thirty asci measured). The very pale brown and slightly curved ascospores were predominantly six-celled with the second cell from the apex always swollen and with the two end cells tapering to a rounded apex and longer than the intermediate cells. Ascospores measured  $18-24 \times 3.5-5\mu$  with an average of  $21.5 \times 4.1\mu$  (thirty-five ascospores measured). Permanent preparations of perithecia, pycnidia and pycnosporangia are deposited in Herb. I.M.I. as No. 15,288. Some material has also been deposited in the Herbarium of the Ministry of Agriculture, Plant Pathology Laboratory, Harpenden.

Desmazières' (1846) diagnosis runs as follows: '*Sphaeria* (caulicola) *nigrans*, Rob. in Herb. S. minuta, sparsa epidermide nigricante tecta. Peritheciis globosis vel sub-ellipticis depressis, albo farctis, basi villis brunneis vestitis. Ostiolo erumpente papillaeformi. Ascis clavatis; sporidiis fusiformibus curvulis, subhyalinis, 5-septatis.

Hab. in vagina Dactyli glomerati. Vere.'

Desmazières also made the following comments: 'Des taches brunes, quelquefois noirâtres, et qui se trouvent au printemps sur la gaine des feuilles sèches du *Dactylis glomerata*...seulement de très petits ostioles ponctiformes et d'un beau noir le percent et s'élèvent au-dessus. Ces périthéciums...n'ont pas plus de  $1/5$  de millimètre de grosseur....Les thèques ont environs de  $1/10$  millimètre de longueur, et s'épaississent insensiblement de la base au sommet...des sporidies,...d'une couleur olive extrêmement pâle, et longues de  $1/50$  de millimètre sur une épaisseur cinq fois moins considérable....Cette espèce curieuse et bien distincte a été récoltée au parc de Lébissey, pres de Caen, au mois d'avril 1843.'

Cesati and de Notaris (1863) made a new combination as follows: '*L[eptosphaeria] nigrans-Sphaeria nigrans* Roberg. in Desmaz. xiii. not. n. 25.

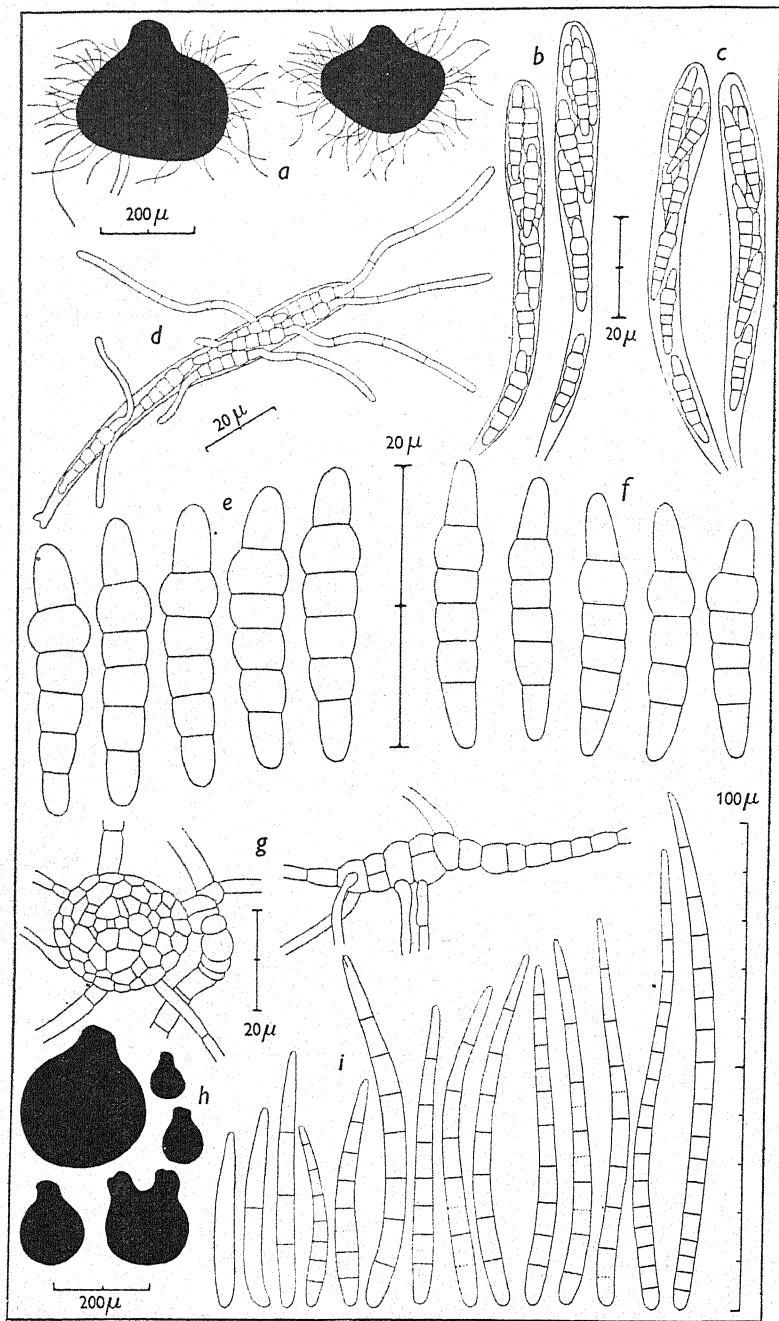


Fig. 1. *Leptosphaeria nigrans* a, b, d, e, g, h, and i were drawn from Herb. I.M.I. No. 15,288; c and f were drawn from French material cited in the text (Slide in Herb. I.M.I. No. 1838). a, perithecia; b and c, asci; d, ascospores germinating within the ascus; e, ascospores drawn in water; f, ascospores drawn in lactic acid; g, primordia of pycnidia drawn from culture on potato dextrose agar; h, pycnidia from culture on potato dextrose agar; i, pycnosporos in various stages of development—from culture on potato dextrose agar.

e specim. a Cl. Lenormand. Berk. et Br. Brit. fung. n. 640. tab xi. fig. 27.' The legitimate citation is therefore *Leptosphaeria nigrans* [(Roberge in Desm.)] Ces. & de Not.

Through the kindness of the Director of the Royal Botanic Gardens, Kew, and of Miss Wakefield, I examined specimens which, if not actually a part of the type collection, are at least authentic for the name *L. nigrans*. This collection, 'Ex Herb. Berk. 1879', consists of five stems with sheathing leaf bases of *Dactylis glomerata* with the following label in a handwriting which has not been identified:

'*Sphaeria Nigrans* Roberg. (Desm 1774)

Sur la gaine des feuilles sèches du *Dactylis glomerata* Caen Roberge.'

Two perithecia measured 180 and 216  $\mu$  in diameter respectively. They appeared black in surface view and possessed brown hyphae, 2.5–4  $\mu$  wide, radiating from the perithecial wall. The epidermis is a little darkened and the papillate neck is only barely visible. Eight asci gave a range of 62–80  $\times$  6.5–8  $\mu$ , whilst ten ascospores measured 16–21  $\times$  3.5–4  $\mu$  and the second cell from the apex was constantly swollen. The ascospores are generally six-celled although one spore had only five cells.

Ascus and ascospore measurements of the Welsh material (Herb. I.M.I. No. 15,288) were made from water mounts of fresh material and those of the French material were made from lactic acid mounts, and this may account for slight differences in measurements. In glycerine jelly, however, the ascospores of I.M.I. No. 15,288 gave a range of only 17.5–21  $\times$  3.5–4  $\mu$ .

Also in Herb. R.B.G. Kew is Desmazières *Crypt. France* No. 1774 issued as '*Sphaeria nigrans* Rob. in herb.' (Slide in Herb. I.M.I. No. 16,409.) This material is authentic for the name *Leptosphaeria nigrans* if not actually part of the original collection by Roberge. The fungus is described as occurring 'sur la gaine des feuilles du *Dactylis glomerata*'. Five perithecia removed from under the darkened epidermis ranged between 220 and 300  $\mu$  in diameter and bore brown hyphae radiating from them. Asci measured 64–90  $\times$  6.5–8  $\mu$  and the ascospores 16.5–19  $\times$  3–3.8  $\mu$ . The ascospores were mostly 5-septate with the second cell from the apex always swollen; occasional ascospores were 4-septate because the third cell from the apex had failed to divide.

The R.B.G. Kew specimen of Karsten's *Fungi Fennica Exsiccata* 962 was also examined (Slide in Herb. I.M.I. No. 15,173). This exsiccatum was issued as *L. nigrans*. The host is not identified but it is graminaceous. A single perithecium was 300  $\mu$  wide and 250  $\mu$  high. The ascospores measure 19–21  $\times$  4–5  $\mu$  and are regularly 5-septate.

In all respects the four specimens mentioned above agree with each other sufficiently to be regarded as one species.

About fourteen ascus and ascospore isolations were made from I.M.I. No. 15,288. Ascospores germinated readily either inside or outside the ascus. All isolations produced pycnidia of the form-genus *Phaeoseptoria* Speg. (see Sprague, 1943) Pycnidia developed by the repeated septation of a hypha to form a spherical initial. Pycnidia in a three-week-old culture on potato dextrose agar varied between 56 and 176  $\mu$  with a mean diameter of 106  $\mu$  (150 measured); one pycnidium, however, reached a diameter of nearly 300  $\mu$ . The pycnidial walls were dark brown to black and fairly

thick. Pycnospores arose from flask-shaped inner wall cells; at first hyaline they became pale brown, brown in mass, very variable in length with seven to sixteen distinct septa. Pycnospores with less septa than seven were probably immature. The following pycnospore measurements were obtained in culture:

Substrate	Age of cultures	Pycnospores measured	Range (in $\mu$ )		Mean ( $\mu$ )	
			Length	Width	Length	Width
Potato dextrose agar	?	100	52.2-84.6	$\times 3-5$	65.4	$\times 3.5$
Potato dextrose agar	?	100	58.5-81.0	$\times 3-5$	67.7	$\times 3.5$
Potato dextrose agar	4 weeks	200	50.4-88.2	$\times 3-5$	69.4	$\times 3.5$
Potato dextrose agar	4 weeks	200	49.5-97.2	$\times 3-5$	72.0	$\times 3.5$
Steamed wheat leaves	?	200	61.2-108	$\times 3-5$	82.8	$\times 3.5$

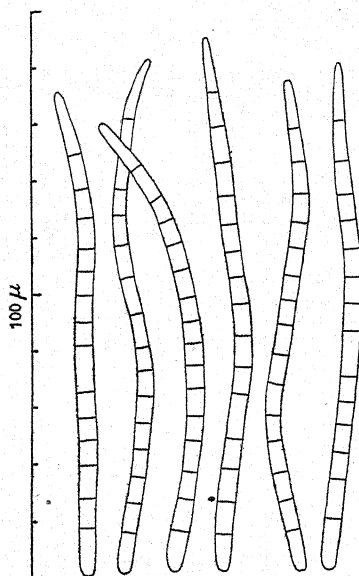


Fig. 2. *Septoria alopecuri* var. *calamagrostidis*. Mature pycnospores from the type collection in Herb. R.B.G. Kew. (Slide in Herb. I.M.I. No. 15,680.)

There is one species of *Septoria* in Grove's *British Stem- and Leaf-Fungi*, the description of which recalls the above pycnidial stage. It is *Septoria alopecuri* Syd. var. *calamagrostidis* Grove, which was described as follows: 'Spores linear, obtuse above, acute below, yellowish,  $40-100 \times 3-4 \mu$ , faintly curved, with 3-13 distinct septa. On dead leaves of *Calamagrostis Epigejos*. Goosehill Woods, Worcs. (Rhodes).' (Fig. 2.)

The type collection in Herb. R.B.G. Kew has been examined (Slide in Herb. I.M.I. No. 15,680). A single pycnidium was mounted in lactic acid tinged with cotton blue and the pale brown pycnospores which were brown in mass measured  $70-97 \times 2.5-4 \mu$  with a mean of  $83 \times 3.1 \mu$  (thirty measurements). The pycnospores are generally curved but sometimes straight, and at maturity possess eleven to seventeen septa. Although the

pycnosporos of the type are very slightly narrower than those obtained in culture the similarity is very great; we have been warned, however, against comparing cultures with material on the host. Nevertheless, I am inclined to query *Septoria alopecuri* var. *calamagrostidis* as a synonym of the *Leptosphaeria nigrans*, and when Grove's variety is refound on the type host and cultured, further investigation will decide whether this assumption is correct or not. In view of the possibility it seems unnecessary to transfer Grove's variety to the genus *Phaeoseptoria* Speg. It is interesting to note that *Leptosphaeria nigrans* has been recorded on *Calamagrostis epigejos* in Denmark (Lind, 1913).

According to Bisby and Mason (1940) the name *Leptosphaeria nigrans* has appeared five times in British literature cited by these authors. It was first recorded for Britain (as *Sphaeria nigrans*) by Berkeley and Broome (1852), who described the fungus as occurring on *Dactylis glomerata* and figured six to eight septate ascospores with a more or less centrally placed swollen cell. Their description and the first British record is based on a collection 'ex Herb Berk' in Herb. R.B.G. Kew labelled thus: '21. *Sphaeria nigrans*, Desm. no. 1774. Batheaston. Feb. 3. 1851.' In this material the perithecia are visible as black pin-points at the surface of the epidermis and there is hardly any darkening of the host tissues. Three perithecia measured 140, 150 and 165  $\mu$  in diameter respectively and bore very few hyphae coming away from the black wall. Asci measured 65-80  $\times$  9-10  $\mu$  and ten ascospores measured 20-23  $\times$  3-4  $\mu$ ; the ascospores are constantly 5-septate with the fourth cell from above always swollen. The three upper cells are each shorter than each of the two lower cells so that the intermediate swollen cell appears centrally placed. This fungus is identified as *Leptosphaeria fuckelii* Niessl and a preparation is so disposed in Herb. I.M.I. as No. 15,327.

Niessl (1887) considered that Berkeley and Broome's description and figures of what they called *Sphaeria nigrans* were based on *Leptosphaeria fuckelii* but Niessl saw no material. Niessl points out the differences between *L. fuckelii*, and *L. nigrans*. He claimed to have examined type or authentic material of *L. nigrans*, viz. Desmazières, *Pl. Cr. de France*, No. 1424 (but not No. 1774), and found the ascospores to be only 16-17  $\times$  2.5-3  $\mu$ , but they were 5-septate with the second cell from the apex swollen.

Fries (1849) claimed *Sphaeria nigrans* as a synonym of his *S. culmicola*. It is impossible to confirm this until type or authentic specimens are available, because Fries described no ascospores.

Cooke (1871) gave an English translation of Desmazières' diagnosis of *S. nigrans* and cited his [*Crypt. France*, Ser. I] No. 1774; Cooke described the fungus as occurring 'on *Dactylis glomerata*. Feb.' I take this to be Berkeley's original record especially because Berkeley's comments are quoted as well, including the description of the ascospores with the 'middle joint' swollen.

Bucknall (1880) recorded '*Sphaeria nigrans* Desm.' from 'Portbury, June, 1879'. Unfortunately, Bucknall's herbarium was destroyed and unless he sent some of his material to some other mycologist and it is still in existence this record cannot be confirmed. There is no material from Portbury in Herb. R.B.G. Kew.

Cooke (1889) listed *S. nigrans* under *Heptameria* Rehm & Thüm. and the



combination may be cited as *Heptameria nigrans* (Rob. in Desm.) Cooke. No specimens were cited.

Massee (1880) listed '*H[eptameria] nigrans*, Desm., Sacc. Syll. 3108; Hdbk. 2716 [Cooke, 1871]. On grass leaves. Shere, Neatishead.' In Herb. R.B.G. Kew there is a specimen ex Herb. M. C. Cooke labelled in Cooke's handwriting: '*Sphaeria nigrans*...immature...Neatishead'. The material consists of two pieces of a graminaceous host which is partly darkened. Four perithecia measured 190–220  $\mu$  in diameter, but no ascospores were found. This British record cannot be confirmed.

The 'Shere' specimen cited by Massee is undoubtedly that found in the *Leptosphaeria nigrans* folder in Herb. R.B.G. Kew (ex Herb. M. C. Cooke), and labelled thus in Cooke's handwriting: 'Dr Capron...Shere...402. Spores same size as *S. culmifraga* but resembling *S. nigrans*.' The *Leptosphaeria* which is present is distinct from *L. nigrans*, but the preparation (Herb. I.M.I. No. 16,053) has not been assigned to a species with satisfaction.

The cultural work was carried out in the laboratory of Mr J. Rees, Advisory Mycologist, University College, Cardiff.

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## STUDIES ON BRITISH CHYTRIDS

## VI. AQUATIC SYNCHYTRIACEAE

By HILDA M. CANTER

*The Freshwater Biological Association, Wray Castle, Ambleside, and  
Department of Botany, Birkbeck College, University of London*

(With Plates VII–XI and 13 Text-figures)

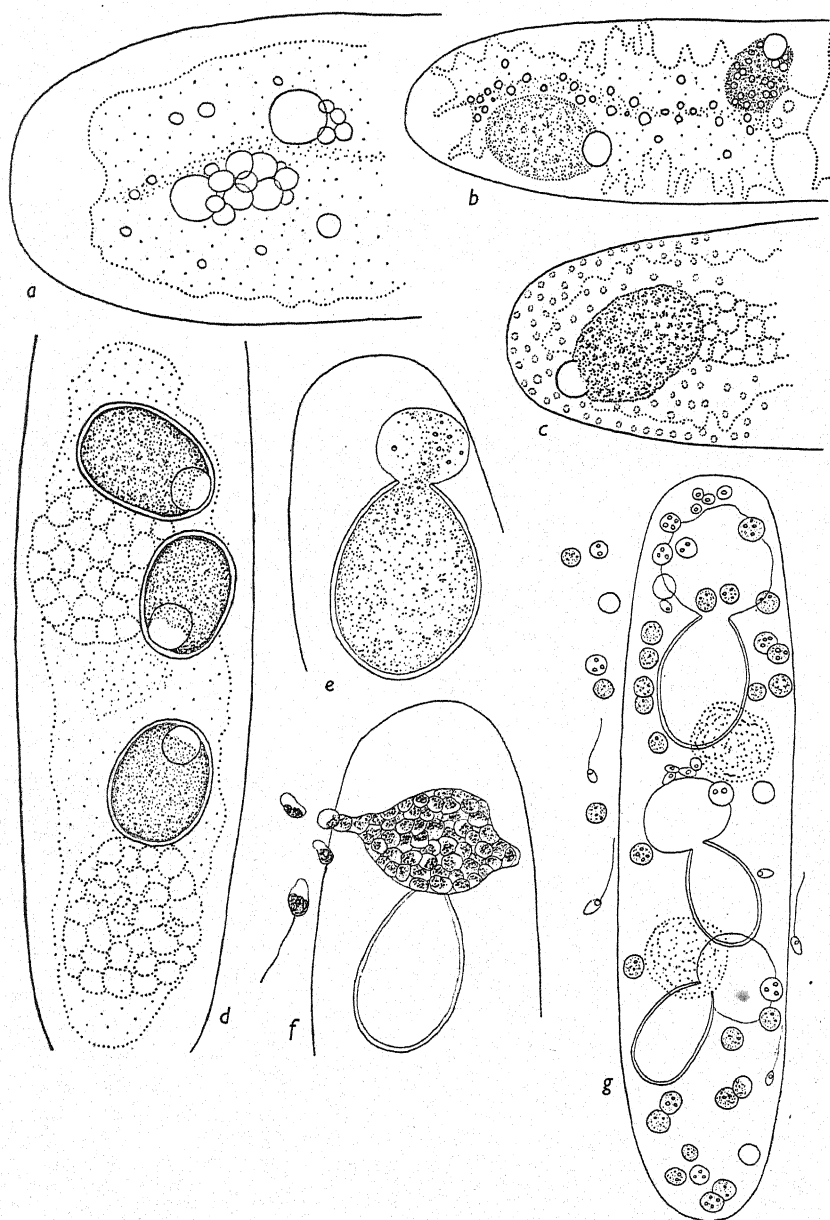
Aquatic representatives of the Synchronytriaceae, apparently widely distributed, have not definitely been described from this country. The older British literature contains many records (Thwaites, 1846–7; Shadbolt, 1852; Smith, 1853) of spiny bodies (asterospheres), causing hypertrophy in members of the Conjugales, which may well have been prosori of *Micromyces* or *Micromycopsis* spp. The writer, while working at the Freshwater Biological Association's Laboratory, Wray Castle, found several species of these genera and a new genus *Endodesmidium*, which seems to be a primitive member of the family.

I. *Endodesmidium formosum* n.gen., n.sp.

In April 1946 what is believed to be a species of a new genus of the Synchronytriaceae was found parasitizing a form of *Netrium oblongum* (De Bary) Lütken with purple sap, in Rusland Moss (a Sphagnum bog), Lancashire.\* In a second collection, made three weeks later, the same chytrid was found on *Cylindrocystis crassa* De Bary, and *C. brebissonii* Menegh. The organism appears to be new to science and a detailed account of its life history on *Netrium oblongum* follows.

The first sign of fungal attack is a disintegration of the host chloroplast, and the accumulation of fatty material along its central axis. The parasite is first visible as a large oil globule by the side of which smaller oil globules accumulate (Text-fig. 1a and Pl. VII, fig. 2). Owing to the dense algal contents the actual fungal protoplasm cannot be distinguished at this stage. With the accumulation of oil globules a naked oval thallus becomes visible (Text-fig. 1b, c, and Pl. VII, fig. 3). The contents of this gradually become more granular, but the large oil globule remains as a characteristic feature. Around this naked thallus a wall is secreted, forming a body equivalent to the prosorus of other aquatic Synchronytriaceae, with the oil globule at the anterior end. The wall is usually coloured a brownish purple, possibly due to the precipitation of the pigment from the purple host sap; it rarely remains colourless and is always smooth. The number of parasites in each host cell varies from one to six, and their position is in no way connected with that of the nucleus. Whether, when more than one parasite is present in a cell, it is due to fragmentation of the fungal cytoplasm, or to separate

\*. This fungus was again collected in April 1947 from the same locality.



Text-fig. 1. *Endodesmidium formosum* n.sp. *a*, very young thalli with conspicuous oil globule and a few smaller globules posteriorly.  $\times 1333$ . *b*, *c*, naked prosori each with a conspicuous anterior oil globule. *b*,  $\times 700$ , *c*,  $\times 1000$ . *d*, three mature prosori.  $\times 975$ . *e*, germinating prosorus.  $\times 975$ . *f*, mature sorus with non-swarming amoeboid swimmers emerging from it; the large oil mass of each swimmer is shaded and one specimen has a short posterior flagellum.  $\times 975$ . *g*, three germinated prosori with dehiscent sori; sporangia in various stages of development and actively motile zoospores both inside and outside the host cell.  $\times 700$ .

infections is unknown. By the time the prosorus is mature the host chloroplasts are reduced to two yellowish oily masses, while the cytoplasm is greyish and much shrunken. The prosori are usually oval and vary from  $16.5 \times 28.5$  to  $10.6 \times 15.6 \mu$ .

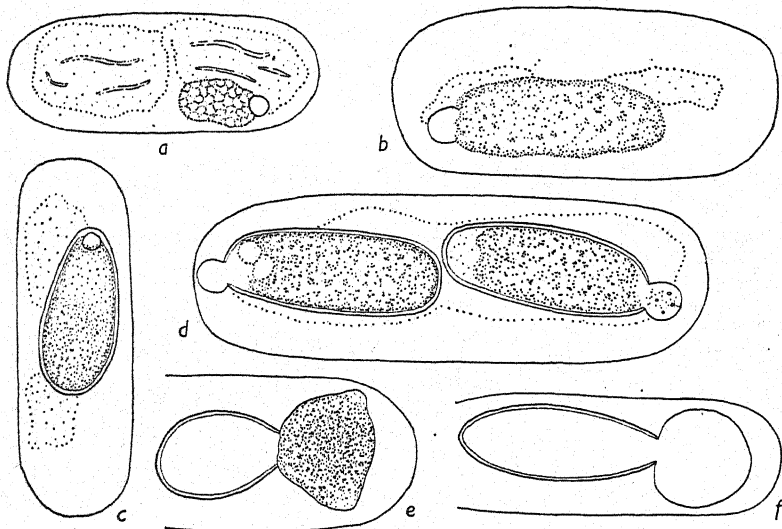
Germination of the prosorus readily takes place. A pore is formed at the anterior end, the oil globule loses its identity, and the contents squeeze out to form a thin colourless smooth-walled vesicle (the sorus) within the host cell (Text-fig. 1e, and Pl. VII, fig. 7). The sorus continues to swell as material gradually passes out from the prosorus leaving a clear area at its base. The mature sori are subspherical and range from  $16-25 \mu$  high  $\times$   $12-21 \mu$  broad. In older specimens which have dehisced and are completely empty, a projection of wall material at the apex of the prosorus is sometimes seen, suggesting that dehiscence of the prosorus is by a lid. The sorus when mature has two oppositely directed lateral papillae, which may or may not pierce the host wall. The soral content divides to form about fifty oily structures,  $3-4 \mu$  in diameter, which ooze singly through the papillae, either into the external medium or into the cavity of the *Netrium* cell (Text-fig. 1f). These bodies are very sluggish, remaining quiescent or exhibiting amoeboid movement; free active motion was never observed. Three specimens only were seen with a single short posterior protoplasmic thread, possibly a flagellum. These bodies (sporangia) are soon all quiescent, the content becomes clearer and from two to five minute oil globules, indicating the position of the zoospores, can be distinguished (Text-fig. 1g, and Pl. VIII, fig. 1). The sporangia liberate from two to five normal chytridiaceous zoospores,  $1 \mu$  in diameter, with a long flagellum. These small sporangia producing a few zoospores are very reminiscent of the cysts arising from the primary zoospores, which are liberated from resting spores in *Allomyces cytogenus* (Emerson, 1941).

The actual fate of the zoospores liberated from the sporangia of *Endodesmidium formosum* is unknown. They swim actively and are no doubt the dispersal phase of this organism. Almost certainly they reinfect the *Netrium*, although empty zoospore cases have never been observed on the outside of the host wall. If, however, the zoospores behave as recorded for *Synchytrium endobioticum* (Schilb.) Percival, by Curtis (1921), then no zoospore case would be visible, since the naked zoospore itself penetrates the host wall.

Several mature prosori were stained for the presence of a nucleus. In five specimens a densely staining area (Pl. VII, fig. 4b), probably the nucleus, was located at the base of the large anterior oil globule. This position is perhaps suggestive of a centre of organization, for in young stages, growth of the prosorus is accomplished by the addition of material behind the large oil globule. From this densely staining area a network of granules extended into the surrounding cytoplasm.

The occurrence of resting spores is very doubtful. After some material had been kept at  $8-11^\circ \text{C}$ . for about five weeks it was examined, and many ungerminated prosori were still present. They were structurally indistinguishable from those already described, except in having perhaps a slightly thicker wall. They germinated after they had been subjected to a temperature of  $19-20^\circ \text{C}$ . for a few days.

As mentioned earlier, this chytrid was also found on *Cylindrocystis crassa* and *C. brebissonii*. Although the actual emergence of the non-swarming zoospores (sporangia) was never observed, the sequence of events followed in its life history are the same as those described above. The prosorus of the chytrid in these desmids was rather longer and narrower, varying from  $15 \times 35$  to  $9 \times 21 \mu$ , and the subspherical sorus from  $13 \times 17$  to  $12.5 \times 13 \mu$ . This form is illustrated in Text-fig. 2 *a-f* and Pl. VII, figs. 5, 6.



Text-fig. 2. *Endodesmidium formosum* n.sp. *a, b*, young naked prosori in *Cylindrocystis*; note conspicuous anterior oil globule. *c*, mature prosorus. *d*, two germinating prosori. *e*, empty prosorus with almost mature sorus; two dehiscence papillae just visible. *f*, dehiscent sorus. *a, c*,  $\times 700$ ; rest,  $\times 975$ .

### *Endodesmidium* n.gen.

Thallus endobiotic, holocarpic, at first naked, later transformed into a smooth-walled prosorus; sorus endobiotic, thin walled, smooth, the content dividing into numerous bodies which emerge through papillae in the external medium or into the cavity of the host; sporangia spherical producing minute zoospores; zoospores posteriorly uniflagellate with a conspicuous oil globule.

### *Endodesmidium* gen.nov.

Thallus endobioticus, holocarpicus, primo sine tunica, demum in prosorum laeve transformatus. Sorus endobioticus, tenuiter tunicatus, laevis, parte interiore in corpuscula numerosa per papillas emergentia partiente. Sporangia sphaerica. Zoosporae minutae, postice uniflagellatae, guttula oleosa distincta praeditae.

### *Endodesmidium formosum* n.sp.

Prosorus oval,  $28.5 \times 16.5$  to  $15.6 \times 10.6 \mu$ , with a smooth, usually purple wall; sorus subspherical,  $16-25 \mu$  high  $\times 21-12 \mu$  broad, having at maturity two oppositely directed dehiscence papillae, the content dividing into

about fifty bodies ( $4\mu$  in diameter) with a conspicuous mass of oil and rarely a single, short posterior flagellum ( $6-8\mu$  long), emerging through the papillae; sporangia spherical,  $4\mu$  in diameter, discharging two to five minute spherical zoospores  $1\mu$  in diameter, with a conspicuous oil globule and long posterior flagellum; movement active swimming; resting spore similar to prosorus, wall slightly thicker.

Parasitic in *Netrium oblongum*, *Cylindrocystis crassa*, and *C. brebissonii*, from Rusland Bog, Lancashire, England, in April 1946.

*Endodesmidium formosum* sp. nov.

Prosorius ovalis,  $28.5 \times 16.5$  ad  $15.6 \times 10.6\mu$ , tunica laevi, plerumque purpurea. Sorus subsphaericus,  $16-25\mu$  altus,  $12-21\mu$  latus, maturitate papillis dehiscentiae duabus oppositis praeditus; pars interior in c. 50 corpuscula partiens; corpuscula  $4\mu$  diam., massa oleosa distincta et rare flagello singulo  $6-8\mu$  longo postice praedita, per papillas emergentia, pigre se moventes. Sporangia sphaerica  $4\mu$  diam.; zoosporae minutae  $1\mu$  diam., guttula oleosa distincta praeditae, postice longe uniflagellatae, libere natantes. Spora perdurans ut prosorius similis, tunica paullo crassiore. Hab. Parasiticus in *Netrio oblongo*, *Cylindrocystide crassa* et *C. brebissonii*, Rusland Bog, Lancashire, Angliae, April 1946.

There seems to be little doubt that this chytrid is a member of the Synchronytriaceae and may possibly represent a primitive condition within the family, where the numerous sporangia emerge from the sorus as relatively large, possibly uniflagellate, amoeboid swarmers, which later encyst and produce a few uniflagellate chytridiaceous zoospores. Scherffel's (1926) investigations on *Micromycopsis cristata*, although not based on continuous observations, are extremely interesting in this connexion. He occasionally found that the sporangia were set free from the soral membrane before maturity of the zoospores. They emerged as relatively large ( $4\mu$  in diameter) uniflagellate (flagellum  $6-8\mu$  long), non-swarming, amoeboid swarmers, with a single large oil globule  $3\mu$  in diameter; they encysted and produced zoospores endogenously. These observations agree with those recorded for *Endodesmidium formosum*, where this is the normal procedure. Scherffel also states that on such rounded portions lying free in the water, which apparently correspond to the amoeboid swarmers, there appear sometimes, two fine thread-like unbranched flagella-processes, which he suggested might be rhizoids. The only structures resembling the latter found by the author were bacteria.

II. *MICROMYCOPSIS FISCHERI* SCHERFFEL

*M. Fischeri* was erected by Scherffel (1926) for an incompletely known fungus, differing from other species of the genus in its smooth, colourless soral wall. A similar organism was found by me during April and May 1947 parasitizing *Tetmemorus brebissonii* (Menegh.) Ralfs, from a path leading to Three Dubs Tarn, Claife Heights, near Sawrey, Lancashire. Although *T. granulatus* Bréb. was abundant, it, together with other desmids, was not attacked.

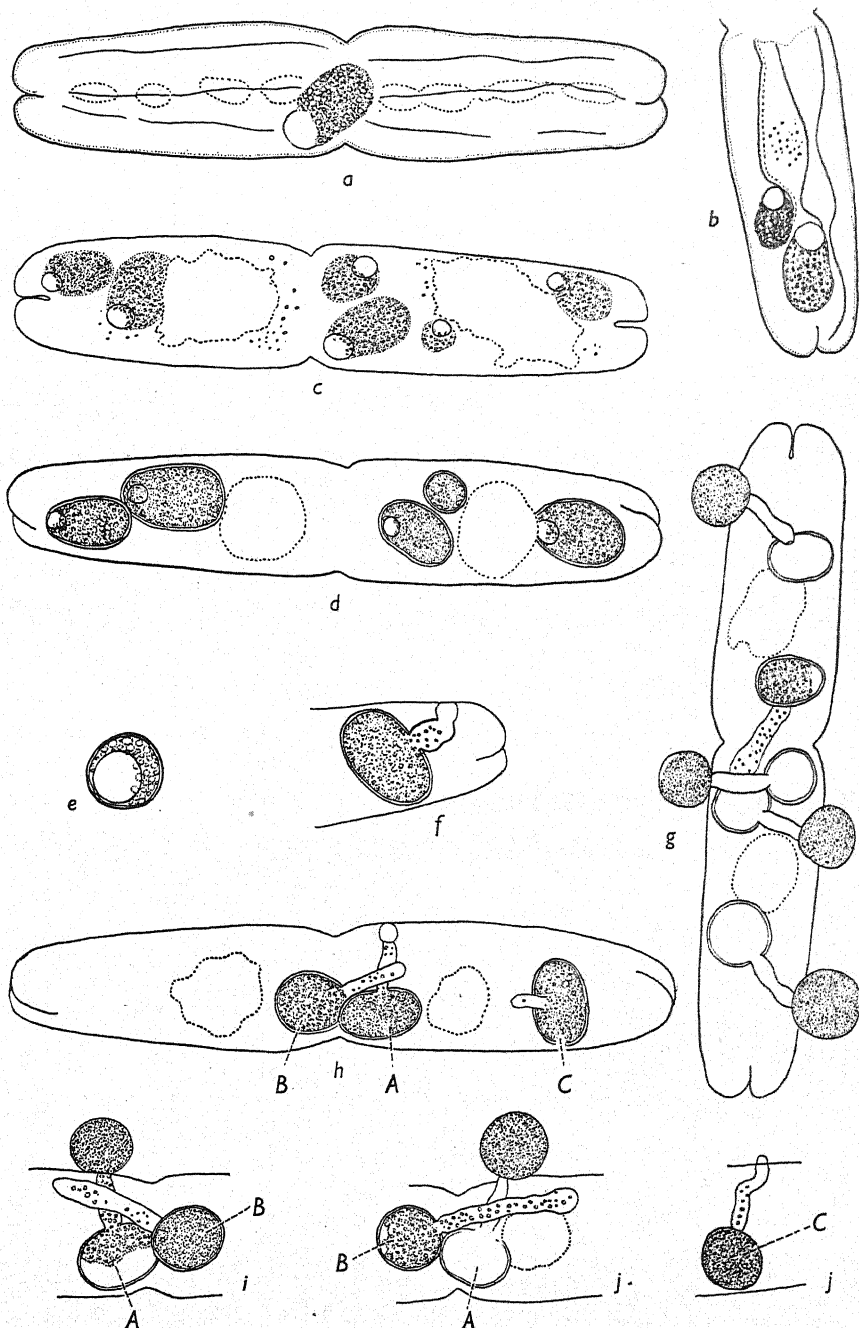
The early stages in development of the prosorius, as well as the mature

prosorus resemble *Endodesmidium* and indeed, until the formation of the sorus, I believed it to be this organism. The fungus is first distinguishable as a large oil globule at one side of which granules appear to accumulate and soon a naked chytrid thallus is apparent (Text-fig. 3*a-c*), which later becomes enveloped in a smooth, colourless wall. The mature prosori are oval ( $12 \times 17$  to  $25 \times 40 \mu$ ) to spherical ( $8-12 \mu$  in diameter); from one to fifteen (rarely thirty) occur in a single cell. Their content is densely granular (appearing brownish in the mass) and the large oil globule is usually distinguishable at one side (Text-fig. 3*d* and Pl. IX, fig. 2).

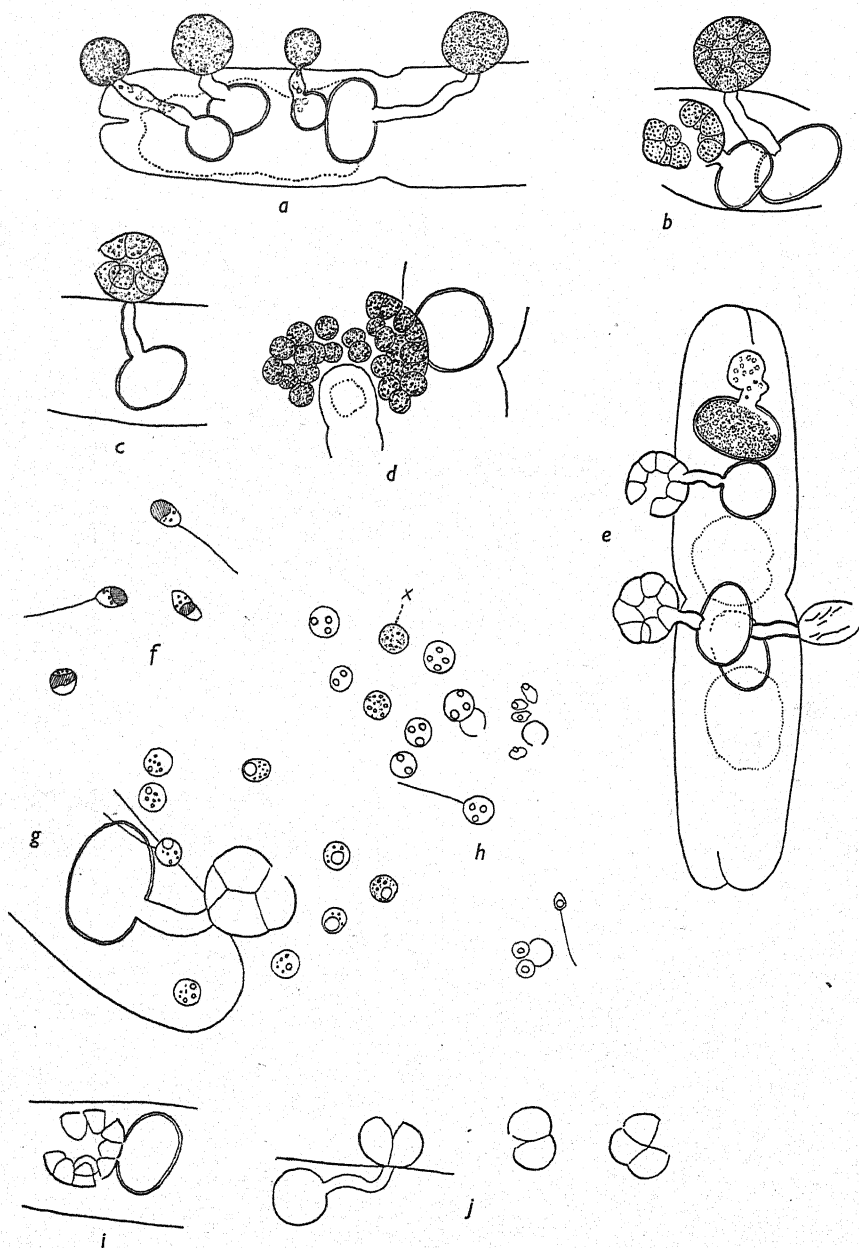
On germination, an exit tube grows from the prosorus to the outer surface of the host wall (rarely extending beyond). The content of the prosorus gradually emerges to form a spherical sorus  $8-20 \mu$  in diameter, with a smooth colourless wall. In early stages the content is hyaline (Text-fig. 3*f, h*), but when all the granules of the prosorus have passed out it in turn appears brown. Endobiotic sori, sessile on the prosori, were sometimes encountered. They differ in no way from the epibiotic ones, and it is considered that only one organism is present. The sorus becomes cut up into sporangia which vary in number according to its size; eight to sixteen in large sori, while small ones have only two to three (Text-fig. 4*j*). The sporangia undergo differentiation; the protoplasm becomes more oily and in each sporangium about five large bodies are formed which emerge singly through a pore to the outside medium. These bodies (primary zoospores),  $4.3 \times 2.4 \mu$ , have a large anterior mass of oil, while posteriorly the cytoplasm is more granular (Text-fig. 4*f*). A single posterior flagellum is present, and although these zoospores show jerky and amoeboid movements they have never been observed swimming freely through the water. After some time they become motionless and spherical, the large mass of oil remaining clearly visible (Text-fig. 4*g*). This mass gradually becomes dispersed and the content is then more granular (Text-fig. 4*h* (*x*)). Finally from two to six spherical oil globules appear in the hyaline protoplasm, each indicating the position of a zoospore. The secondary zoospores ( $2 \mu$ ) emerge through a pore and swim actively through the water by means of a posterior flagellum  $7.6 \mu$  long. Resting spores were not observed. This remarkable behaviour has been observed on several occasions and there is no doubt that it represents the normal course of events in this species.

Scherffel's investigations on the flagellate primary sporangial stage in *Micromyopsis cristata* Scherffel have already been referred to in connexion with *Endodesmidium* (p. 73). The organism described above lends further support to these observations. However, the number of zoospores emerging from the primary sporangia in *Micromyopsis cristata* remains unknown. The phenomenon of two sporangial and zoosporic phases is unique in the chytrids and among other aquatic fungi most nearly resembles that found in the Cystogenes group of *Allomyces* (Emerson 1941). Here the resting spores on germination produce large sluggish biflagellate zoospores which soon encyst and later give rise to four posteriorly uniflagellate zoospores. A similar condition is seen in the diplanetism of members of the Saprolegniaceae. The sporangium in *Saprolegnia* may, perhaps, be considered a sporangiosorus, each zoospore giving rise to a single sporangium (the encysted primary zoospore), which later liberates but a single zoospore.





Text-fig. 3. *Micromycopsis fischeri*. a-c, naked prosori. d, mature prosori. e, small spherical prosorus with large oil globule. f, very early stage in germination of prosorus. g, empty prosori with discharge tubes and sori. h, germinating prosori 4 p.m. 8 May 1947. i, A and B, 18 hours later. j, A, B, C, 42 hours later. e,  $\times 1050$ ; the rest,  $\times 500$ .



Text-fig. 4. *Micronymopsis fischeri*. a, prosori with sori; in one content is beginning to segment. b, epi- and endo-biotic sorus of sporangia. c, sorus of sporangia, soral wall split. d, squashed sorus of sporangia. e, germinating prosorus and three empty sori. f, primary zoospores, anterior mass of oil cross-hatched. g, empty sorus with recently encysted zoospores. h, various stages in maturation of secondary sporangia and dehiscence of zoospores. i, empty endobiotic sorus. j, small sori with two to three sporangia. a-e, i,  $\times 500$ ; f-h,  $\times 1050$ ; j,  $\times 800$ .

At present, the genus *Micromycopsis* is separated from *Micromyces* on the following characters: position of the sorus (epi- or endobiotic), nature of the soral wall (spiny or smooth), size of the sporangia, and number of zoospores produced in each sporangium. *Micromycopsis fischeri* clearly shows that these are not altogether trustworthy characters on which to base generic distinction. While the majority of the sori are formed at the end of a discharge tube as is characteristic for *Micromycopsis*, sometimes they may be sessile on the prosorus resembling *Micromyces*. Secondly, in *Micromycopsis* the sorus wall is characteristically brown and granular or spiny whereas, *M. fischeri* has the smooth colourless wall of a *Micromyces*. It is possible that many more intermediate types will be discovered, such as a *Micromyces* possessing a brown spiny sorus wall, and as suggested by Sparrow (1932) the genus *Micromycopsis* may be found to be superfluous.

Again, early stages in the development of *M. fischeri* resemble those found in *Endodesmidium* and perhaps in the future a series of species will be found so closely linking all these genera as to make any distinction between them artificial.

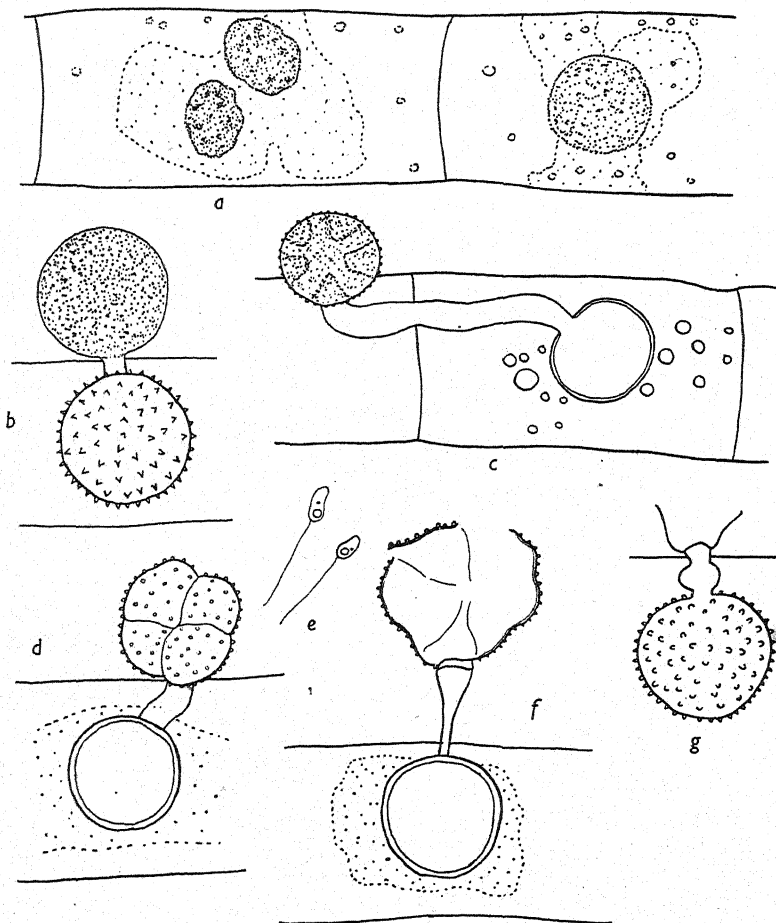
### III. *Micromycopsis intermedia* n.sp.

This fungus occurred on two species of *Zygnema* in the Sphagnum bog, bordering the north side of Blelham Tarn, Lancashire, during April 1946. Hypertrophy of the host cell is rarely seen, but the host chloroplast, and cytoplasm are usually reduced to a blackish mass surrounding the mature prosorus. The young thallus is first visible as a naked greyish oily mass of protoplasm, often situated near the centre of the cell (Text-fig. 5a). This thallus enlarges, becomes spherical, and invested by a wall to form a prosorus (10–17.8  $\mu$  in diameter), the larger specimens generally occurring singly, and the smaller in groups in the host cell. The prosoral contents are oily, and the thick brown wall may be spiny, granular or smooth (Text-fig. 5b, c). The spines are generally distributed in contrast to those of *Micromycopsis cristata* Scherffel (Text-fig. 13a, b). The prosorus on germination produces a tube which pierces the wall of the host and may extend to a varying distance beyond the host wall; at its apex, and separated by a cross-wall, a sorus develops (Text-fig. 5f, g and Pl. X, fig. 10). The spherical sorus is approximately equal in size to the prosorus and has a yellowish brown wall covered with minute blunt spines. The soral wall splits into four or six parts, exposing the same number of hyaline sporangia, each broadly triangular with a rounded base. The sporangia set free from twenty to thirty oval zoospores each with a conspicuous posterior oil globule and a smaller globule at the side. These swim actively with a single posterior flagellum. Neither reinfection of the host by the zoospores nor resting spores was seen.

Scherffel (1926) has reported that sometimes the sporangia in *M. cristata* emerge from the soral wall as relatively large uniflagellate amoeboid swimmers which then encyst and produce endogenously a few zoospores. In the species here described only the typical non-flagellate sporangial stage is known.

*M. intermedia* differs from all hitherto described species of the genus in that the soral wall appears to split somewhat radially into as many parts

as there are sporangia. The latter are relatively few in number, broadly triangular, and liberate many zoospores. In these characters *M. intermedia* more nearly approaches the genus *Micromyces*, and in view of this it is decided to erect a new species for the chytrid here described, namely



Text-fig. 5. *Micromyopsis intermedia* n.sp. a, young naked prosori in *Zygnema*. b, germinated spiny prosorus with sessile immature sorus. c, smooth-walled prosorus with long discharge tube and sessile sorus showing signs of division into sporangia. d, smooth-walled prosorus with sorus of four sporangia. e, zoospores. f, non-sessile dehiscent sorus. g, prosorus with swollen exit tube. e,  $\times 1300$ ; the rest,  $\times 1000$ .

*M. intermedia*. It may also be noted that the varying position of the sorus in relation to the host wall in *M. intermedia* suggests that this is not necessarily a trustworthy specific character in the genus *Micromyopsis* (cf. *M. cristata* Scherffel, and *M. zygnaeicola* Cejp).

**Micromycopsis intermedia** n.sp.

Prosorus spherical,  $10.7-17.8\mu$  in diameter, wall brown, spiny, granular or smooth; sorus epibiotic, sessile on the host wall or at some distance from it; spherical, wall yellowish brown, covered with minute blunt spines, splitting into four to six parts to expose the same number of broadly triangular sporangia; zoospores twenty to thirty, oval ( $3.5 \times 1.5\mu$ ) with a conspicuous oil globule, and smaller refractive globule at the side. Resting spores unknown.

In *Zygnema* spp. Blelham Bog, Wray Castle, England.

*Micromycopsis intermedia* sp. nov.

Prosorus sphaericus,  $10.7-17.8\mu$  diam., tunica brunnea, aculeata, granulosa vel laevi. Sorus epibioticus, sessilis vel pedicellatus sphaericus, tunica flavo-brunnea, aculeis minutis obtusis ornata, in 4-6 partes ita fissa ut sporangia aequinmera late triangularia monstret. Zoosporae 20-30, ovaes,  $3.5 \times 1.5\mu$ , guttula oleosa distincta et guttula refractiva minore praeditae. Sporae perdurantes ignotae.

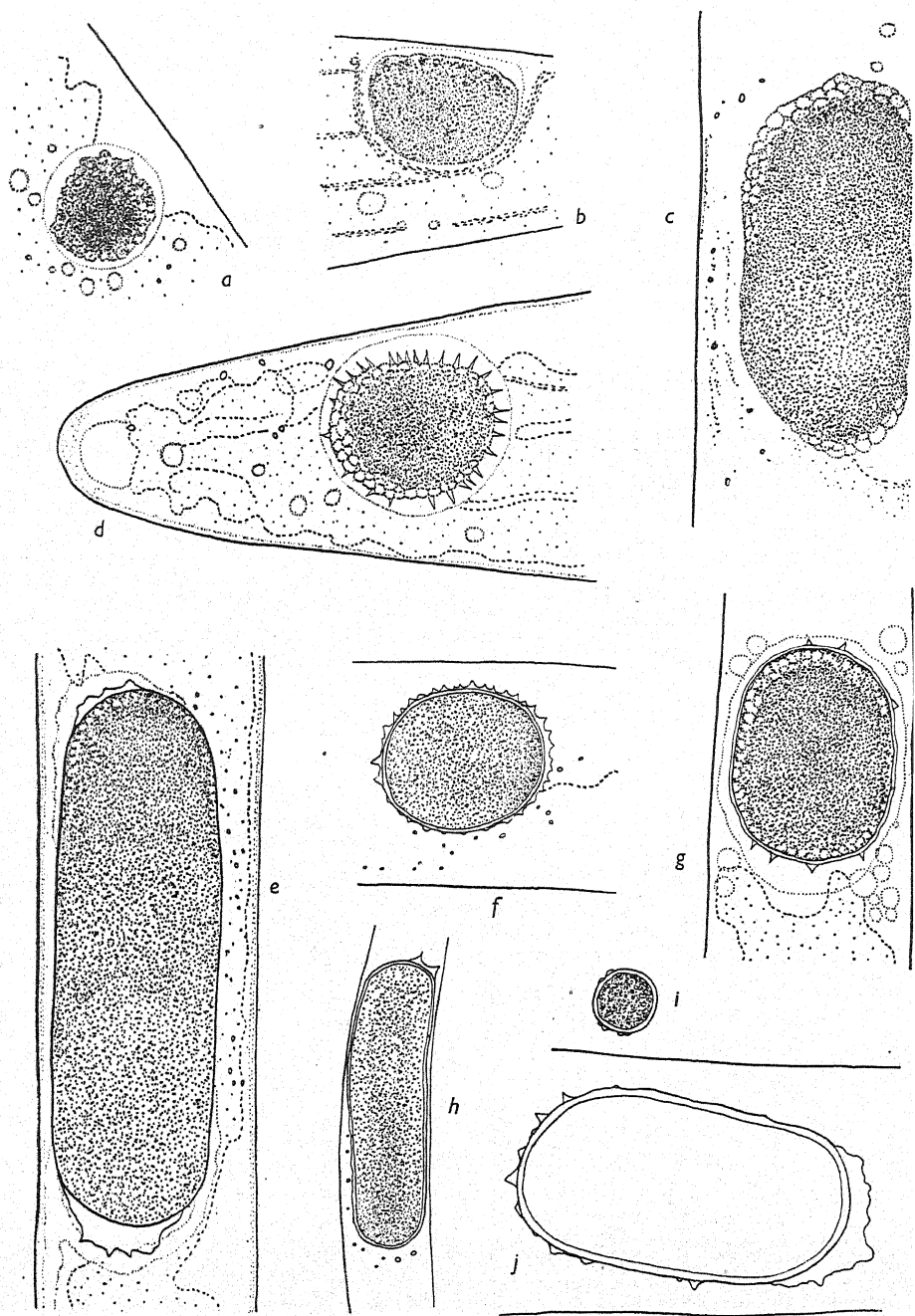
Hab. in *Zygnemata* spp., Blelham Bog, Wray Castle, Angliae.

**IV. Micromycopsis mirabilis** n.sp

This interesting species occurred in *Closterium lunula* Ehrenb., *C. diana* Ehrenb., *C. costatum* Corda., *C. kutzingii* Bréb., and *Closterium* sp. in a collection from a sphagnum pool on the northern edge of Blelham Tarn in January 1947. Although other members of the Conjugales were present, they were not attacked.

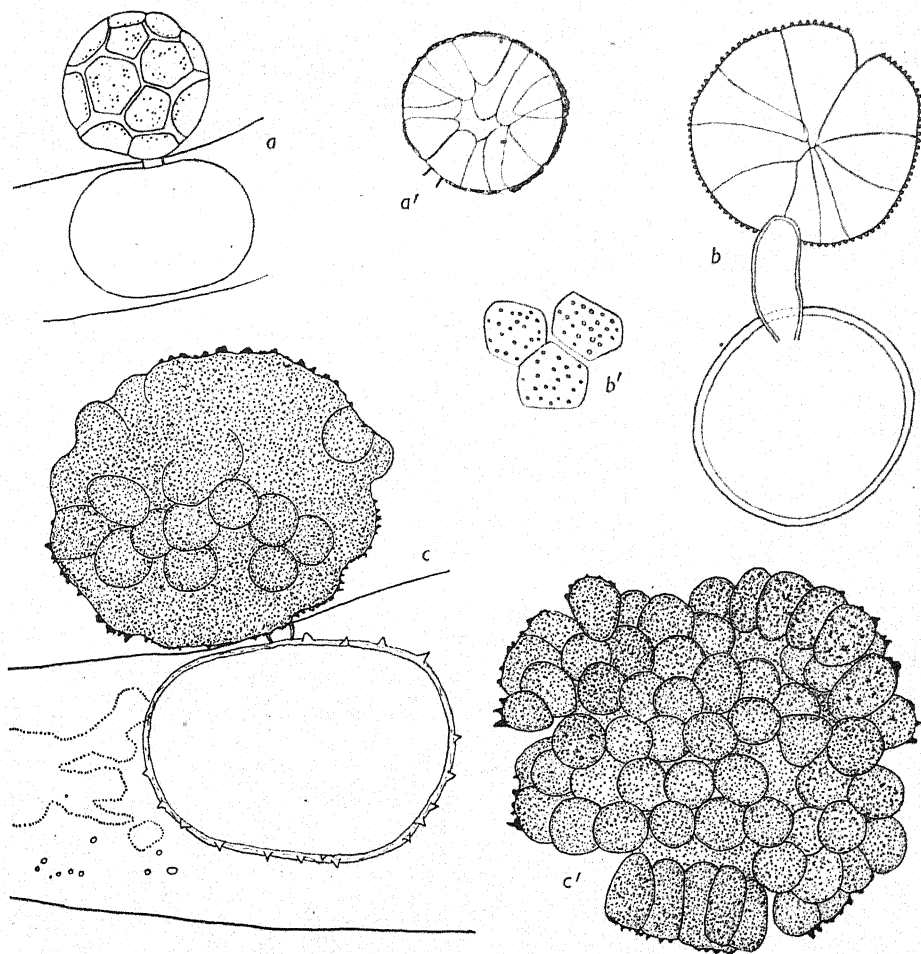
The young naked prosorus consists of a dense mass of globules, appearing black under the microscope, surrounded by a hyaline area (Text-fig. 6a, b). Before the prosorus becomes walled the spines are laid down as naked protoplasmic structures (Text-fig. 6d, and Pl. XI, fig. 2). The mature prosorus has dense contents giving it a blackish appearance and a hyaline wall which sometimes appears to consist of two layers. The outer bears the broad-based spines which may surround the prosorus. If, however, the latter is very elongated and the side walls are closely adpressed to the host wall (e.g. in a narrow *Closterium* sp.), then the spines may be visible only on the end walls, sometimes exhibiting an irregular form (Text-fig. 6g). The inner layer of the prosoral wall is smooth. In old empty prosori the spines are difficult to see and often seem to disappear completely. The prosori vary in shape and size and tend to be smaller when several occur together; seventeen have been found in a single *Closterium*. The prosori can be divided into three classes: the very elongate,  $57 \times 112$  to  $20 \times 75\mu$ ; less elongate,  $55 \times 67$  to  $12.8 \times 21\mu$  and subspherical,  $35 \times 37$  to  $10 \times 12\mu$ .

It may be that some of these bodies related to prosori are in actual fact resting spores. Isolated specimens, having been liberated after disintegration of the host cell, were seen in the decaying algal material and only three germinated prosori were found. The prosorus germinates by a short, thick-walled exit tube and produces a sorus immediately outside the alga.



Text-fig. 6. *Micromycopsis mirabilis* n.sp. *a-c*, naked prosori. *d*, naked prosorus with developing spines surrounded by a conspicuous hyaline area. *e-j*, mature prosori showing variation in size and degree of development of the spines. All  $\times 500$ .

The sorus is spherical, roughly equal in size to the prosorus with a yellowish brown wall covered with small blunt spines, and at maturity contains ten to one hundred broadly triangular zoosporangia (Pl. XI, fig. 3). The sorus, when viewed from above, shows polygonal areas which mark the position



Text-fig. 7. *Micromycopsis mirabilis* n.sp. *a*, empty prosorus and sorus, outlines of zoosporangia seen in surface view. *a'*, the same in optical section.  $\times 500$ . *b*, prosorus and sorus in optical section. *b'*, part of soral wall seen in surface view.  $\times 1050$ . *c*, very large prosorus with immature sorus; lines of development of a few zoosporangia are visible.  $\times 500$ . *c'*, the same rather flattened a day later.  $\times 500$ .

of the zoosporangia (Text-fig. 7*a*). Infection of the *Closterium* by the zoospores was not seen, nor could any empty zoospore cases be found.

In the same collection one specimen of a *Micromycopsis* sp. was observed on *Tetmemorus granulatus* (Bréb.) Ralfs. The prosorus was ornamented with granules exhibiting a somewhat spiral arrangement and the sorus possessed a yellowish spiny wall (Pl. XI, fig. 4).



Although incompletely known, these two organisms suggest that there are many forms yet to be discovered. They also, with *Micromycopsis intermedia*, indicate that the zoosporangia in this genus (hitherto in *M. cristata* and *M. zygneticola* known to be usually spherical and to produce a few zoospores) may more closely resemble those known in *Micromyces* where they are broadly triangular, radially arranged and liberate numerous zoospores.

***Micromycopsis mirabilis* n.sp.**

Prosorus (resting spore?) very elongate,  $75 \times 20$  to  $112 \times 57 \mu$ ; less elongate,  $21 \times 12.8$  to  $67 \times 55 \mu$  or spherical,  $12 \times 10$  to  $37 \times 35 \mu$ ; wall thick, hyaline, outer layer with broad-based spines. Sorus epibiotic, spherical,  $28-80 \mu$  in diameter, wall yellowish brown covered with small spines, formed at the end of an exit tube and at maturity containing ten to one hundred triangular zoosporangia  $13-21 \mu$  high  $\times 7-14 \mu$  broad at the base. Zoospores not observed.

Parasitic in *Closterium lunula* Ehrenb., *C. diana* Ehrenb., *C. costatum* Corda, *C. kutzingii* Bréb., and *Closterium* sp. in a Sphagnum pool bordering the northern edge of Blenheim Tarn near Wray Castle, England.

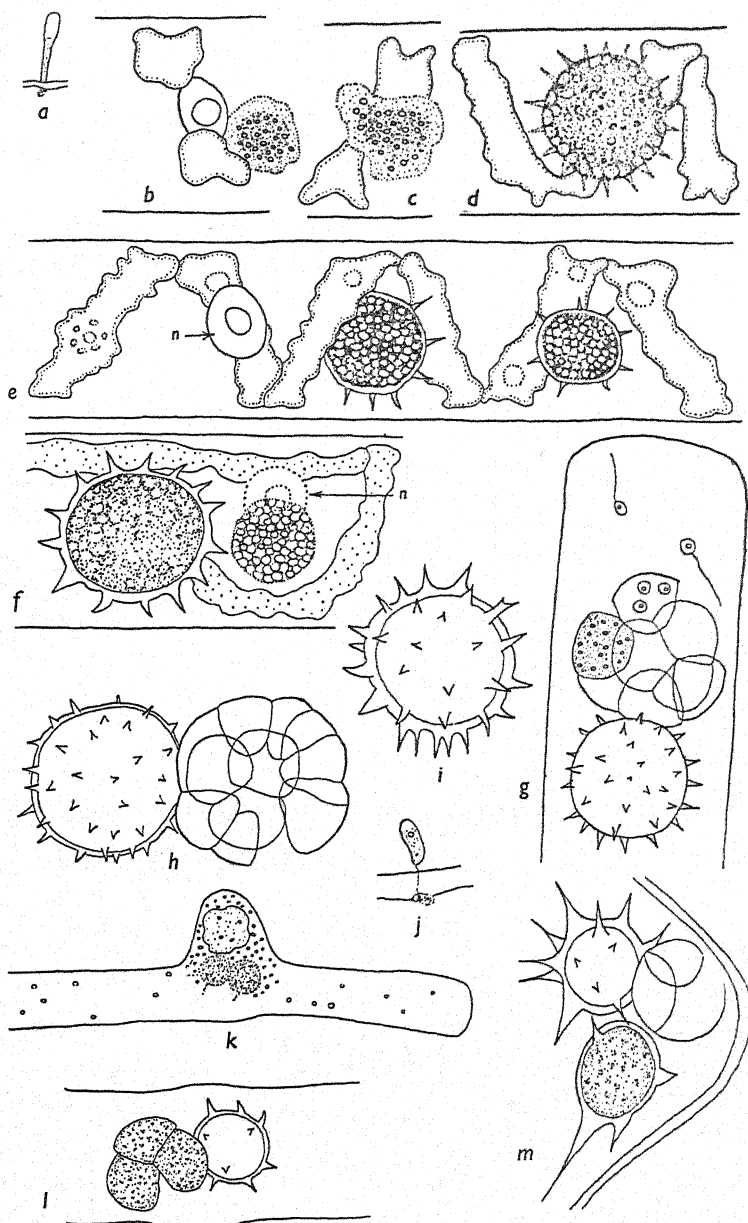
*Micromycopsis mirabilis* sp. nov.

Prosorus (vel spora perdurans?) praelongus  $75 \times 20$  ad  $112 \times 57 \mu$ , vel brevior  $21 \times 12.8$  ad  $67 \times 55 \mu$ , vel sub-globosus  $12 \times 10$  ad  $37 \times 35 \mu$ , tunica crassa, hyalina, aculeis basi crassis ornata praeditus. Sorus epibioticus globosus,  $28$  ad  $80 \mu$  diam., tunica flavo-brunnea, aculeis minutis ornata praeditus; in maturitate  $10$  ad  $100$  sporangia triangularia,  $13-21 \mu$  alta, basi  $7-14 \mu$  lata includens. Zoosporae ignotae. Hab. Parasiticus in *Closterio lunula* Ehrenb., *C. diana* Ehrenb., *C. costatum* Corda, *C. kutzingii* Bréb., et *Closterio* sp.; Blenheim Tarn prope Wray Castle, Angliae.

V. *MICROMYCES ZYGOGONII* DANGEARD

*Micromyces zygogonii* Dangeard, described from many parts of the world, appears to be the commonest species of this genus. It occurred on *Spirogyra* sp. in the Clay Pond, Wray Castle in April 1946, but did not attack *Mougeotia* sp. and *Closterium* spp. which were also present. The zoospore encysts on the surface of the host cell, and its content passes in as a naked mass of protoplasm (Text-fig. 8a), leaving an empty zoospore case on the outside. This naked protoplast, either by its own amoeboid movements or by streaming of the host cytoplasm, reaches a position near the nucleus. This apparent attraction of young thalli to the host nucleus was also noted by Couch (1931, 1937). The young thallus is clearly distinguishable from the host content as a dense, greyish mass of protoplasm with numerous highly refractive globules grouped towards the centre. The peripheral region is finely granular and amoeboid, pseudopodia being constantly formed and retracted (Text-fig. 8b, c). The globules in the centre, however, do not alter their position.

The naked thallus (Pl. X, fig. 1) develops into a spiny walled prosorus. The spines appear before the wall is laid down as naked granular strands



Text-fig. 8. *Micromyces zygogonii*. *a*, contents of encysted zoospore passing into host cell. *b*, *c*, young naked amoeboid thalli. *d*, spherical naked thallus, spines beginning to develop. *e*, two mature prosori. *f*, mature prosorus and young naked prosorus adpressed to the nucleus which is represented by a dotted line. *g*, empty prosorus with a sorus of sporangia, one undehisced; two flagellate zoospores figured. *h*, empty prosorus and sorus of sporangia. *i*, thick-walled spiny resting spore. *j*, *M. petersenii* Scherffel contents of zoospore passing into *Mougeotia* cell. *k*, hypertrophied host cell with two naked prosori above which is the shrunken chloroplast. *l*, short spined prosorus with developing sorus. *m*, prosori with well-developed spines. *a*, *b*, *f*, *h*, *l*,  $\times 1000$ ; *c*, *d*, *g*, *m*,  $\times 975$ ; *i*, *j*,  $\times 1333$ ; *k*,  $\times 375$ . *n*=host nucleus.

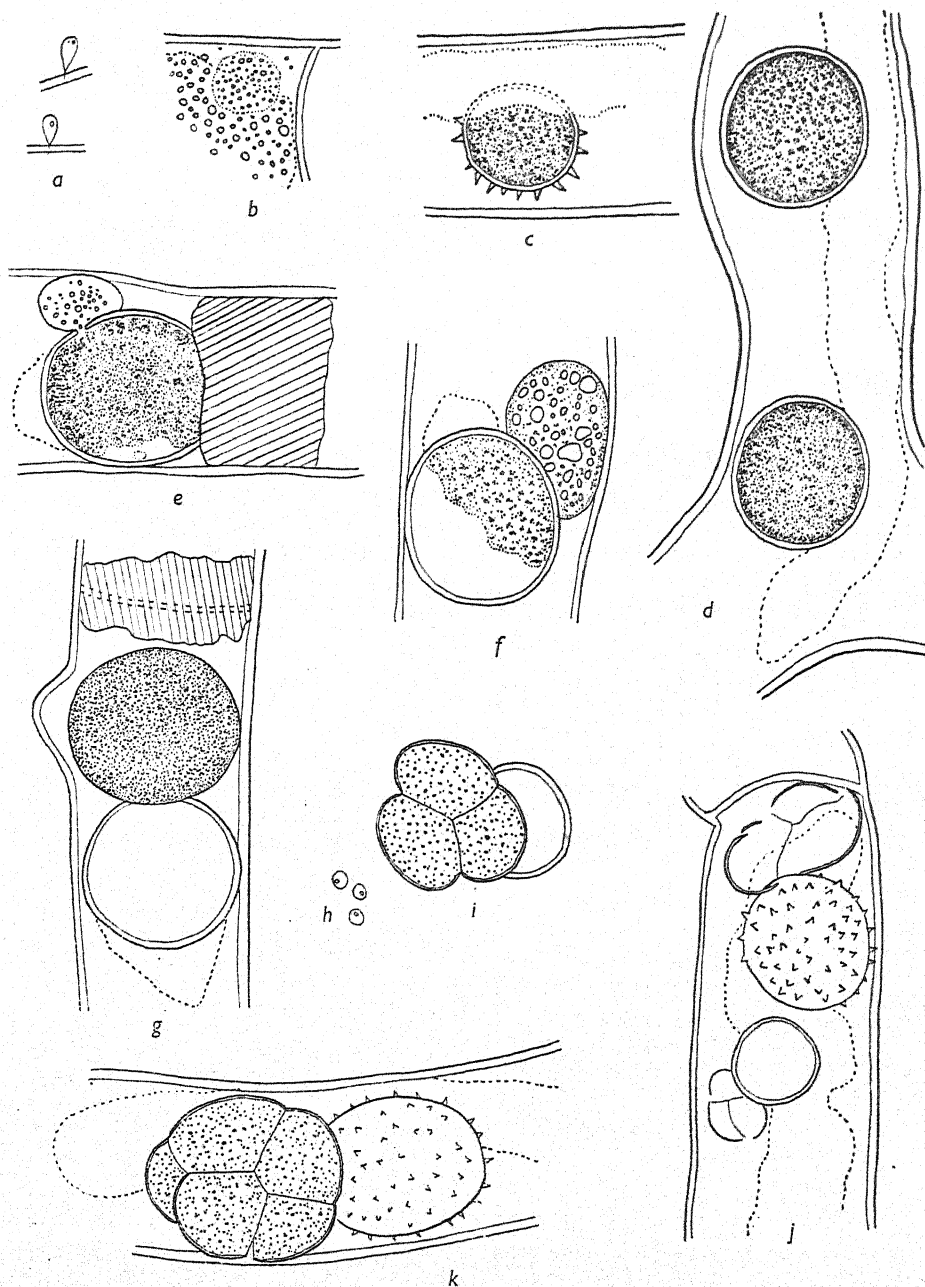
of protoplasm (Text-fig. 8*d*) which later become walled. Couch (1931) observed similar granular strands, mostly connected with the pyrenoids of the host cells, but this connexion was not observed in the material from the Lake District. The mature prosorus is spherical,  $13.4\text{--}17\mu$  in diameter, with a colourless wall bearing numerous sharply pointed spines about  $3.5\mu$  long (Text-fig. 8*e, f*). By the time the prosorus is mature the algal chloroplast shows signs of disintegration, but the distinct hypertrophy of the host cells so commonly recorded (cf. Dangeard, 1889; Huber-Pestalozzi, 1931; Heidt, 1937) was not seen. The prosorus germinates to give a spherical thin-walled sorus,  $13\text{--}17\mu$  in diameter. Zoosporangia are delimited within the sorus and its membrane splits into about eight portions each corresponding to one zoosporangium (Text-fig. 8*g*, and Pl. X, fig. 2). The zoosporangia are  $7\text{--}9\mu$  in diameter, broadly triangular with a rounded base each liberating about twenty zoospores. The zoospore is oval to spherical,  $2\mu$  in diameter, with a single oil globule, and posterior flagellum.

Resting spores (Text-fig. 8*i* and Pl. X, fig. 3) appeared as the *Spirogyra* became moribund, and were often seen in empty host cells whose contents had been ingested by Protozoa. The resting spore,  $11\text{--}15\mu$  in diameter, resembles the prosorus except for its thicker brown wall, and on germination functions as a prosorus.

Later, in January 1947, a species of *Micromyces* was found in a temporary mud pool, bordering the northern edge of Blelham Tarn Sphagnum bog, near Wray Castle, which differs in only very minor respects from *M. zygonii*. It appears to be highly specific to one species of *Mougeotia* and does not attack *Spirogyra* and *Zygnema* spp. Hypertrophy of the host cell is not always produced, but in nearly every infected cell numerous colourless globules collect at the septa, forming a greyish mass which subsequently turns black (Text-fig. 9*g*).

The young stages in development of this form are similar to those previously described (Text-fig. 9*a, b*). The mature prosori (Text-fig. 9*c, d*) are spherical to subspherical,  $6\text{--}22\mu$  diameter, with a thick hyaline, smooth or spiny wall. The contents are densely granular, and give a brownish colour to the prosorus. The smooth-walled sorus (Text-fig. 9*g-k*) is approximately equal in diameter to the prosorus and at maturity the contents divide to form four to eight broadly triangular sporangia,  $12\text{--}15\mu$  high  $\times$   $8\text{--}3\mu$  broad, containing numerous uniguttulate, posteriorly uniflagellate zoospores,  $2\mu$  in diameter.

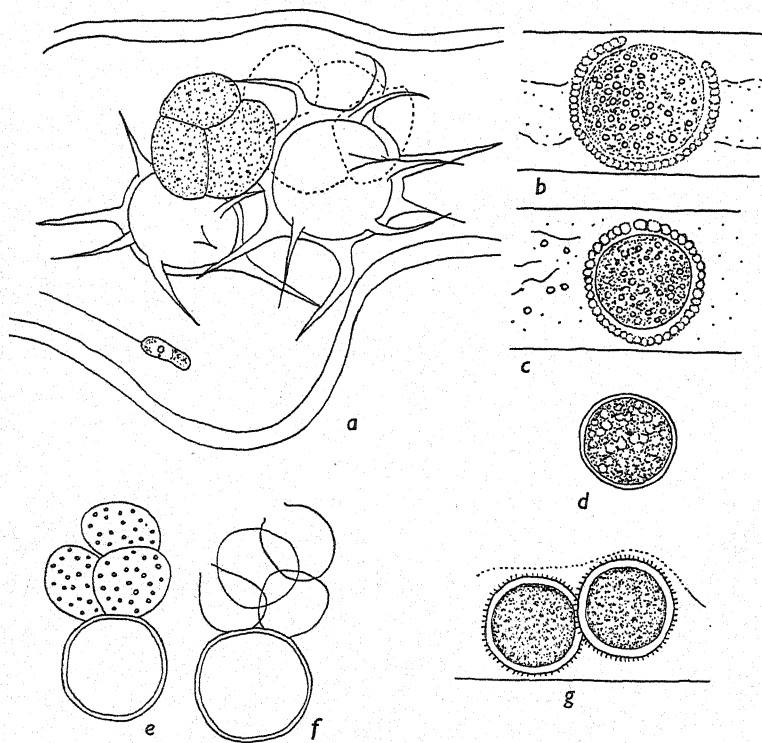
This fungus differs from *Micromyces zygonii* only in the presence of smooth-walled as well as spiny prosori, and in the characteristic blackening of the septa of the host cell. There is as yet no clear idea of trustworthy taxonomic characters for use in specific distinction, and for the present this fungus is included in the species *M. zygonii*. It is very probable that this is not the same fungus as occurs in *Spirogyra* in the Clay Pond, since investigations seem to indicate that the majority of aquatic Synchytriaceae are highly specific to their particular hosts. It may be found in the future that many forms with similar morphological characters can only be distinguished by physiological differences.



Text-fig. 9. *Micromyces zygogonii* Dangeard. *a*, encysted zoospores. *b*, naked prosorus. *c*, spiny walled prosorus. *d*, two smooth-walled prosori in hypertrophied host cell. *e-g*, stages in development of sorus. *h*, zoospores. *i-k*, empty prosori with immature and dehiscent sori. All  $\times 1050$ . Blackened region surrounding the septa in *e* and *g* cross-hatched.

VI. *MICROMYCES PETERSENII* SCHERFFEL

A form similar to *Micromyces zygogonii* was found in Blelham Bog, Wray Castle, causing hypertrophy of *Mougeotia* sp. The prosorus is spherical, 10–15  $\mu$  in diameter, with a thick colourless wall, and usually possesses a few long spines, 6–12  $\mu$  (Text-figs. 8*m*, 10*a*, and Pl. X, fig. 4); rarely a specimen was observed with short spines 2  $\mu$  long (Text-fig. 8*l*). The sorus divides into four sporangia about 7  $\mu$  in diameter which liberate from twenty to



Text-fig. 10. *a*, *Micromyces petersenii* Scherffel prosori and sori inside an hypertrophied *Mougeotia* cell; one zoospore is figured.  $\times 1333$ . *b*, *M. laevis* n.sp. young naked spherical prosorus with an incomplete halo of host granules in *Mougeotia* sp.  $\times 975$ . *c*, almost mature prosorus with halo of granules.  $\times 1000$ . *d*, mature prosorus.  $\times 1000$ . *e*, germinated prosorus with sorus of sporangia; each oil globule indicates the position of a zoospore.  $\times 975$ . *f*, prosorus with a sorus of empty sporangia.  $\times 975$ . *g*, probable resting spores.  $\times 1000$ .

thirty zoospores. The zoospores (Text-fig. 10*a*) are oval,  $5.5 \times 1.5 \mu$ , with a single oil globule, and sometimes a minute shining granule. The zoospores usually move actively, but sometimes they become amoeboid. Resting spores were not observed.

This chytrid, although resembling *Micromyces zygogonii*, has zoospores of a very different size. Scherffel (1926) describes *M. petersenii* on *Mougeotia* sp. which possesses large fusiform ( $6 \times 2 \mu$ ) zoospores, but in this species the prosorus has short conical spines sparingly dispersed over its surface. In

view of the large zoospores it is decided to refer the variety here described to *Micromyces petersenii* rather than to *M. zygonii* in spite of the difference in length of the spines.

#### VII. *Micromyces laevis* n.sp.

The third species of *Micromyces* was found growing on *Mougeotia* sp. in the Clay Pond, Wray Castle, in April 1946. As described for other species, the zoospore settles on the host wall, and its contents pass inside, leaving an empty case on the surface of the alga. Often beneath these empty cases there is a thickening of cell-wall material presumably stimulated by the fungal attack. The young naked thalli are usually situated near the middle of the host cell. Swelling of the latter was not seen and it was difficult to decide if the parasite caused infected cells to elongate, as they normally varied greatly in length. During the development of the thallus a partial or complete halo of colourless host granules often collects around the thallus (Text-fig. 10b, c, and Pl. X, fig. 8). Dangeard (1889, pl. 2, fig. 7) shows a similar halo in *Micromyces zygonii*. The mature prosorus has a smooth colourless wall, oleaginous contents and varies from 13 to 18  $\mu$  in diameter (Text-fig. 10d, and Pl. X, fig. 7). When many prosori develop in the same host cell they tend to be smaller (7.6–11  $\mu$ ). On germination a spherical smooth-walled sorus, of the same size as the prosorus, is produced. The content becomes divided to form from four to eight sporangia, and the sorus wall splits into as many parts, allowing the sporangia to separate slightly. Many oval zoospores, 1  $\mu$  in diameter, with a single oil globule and posterior flagellum, are liberated from each sporangium through an apical pore (Text-fig. 10e). One interesting specimen was observed in which the sorus formed outside the *Mougeotia* cell instead of endobiotically, as is characteristic for the genus *Micromyces*. Resting spores were not recognized with certainty. Thalli, 12.7  $\mu$  in diameter, similar to prosori but with a brown wall, which in three specimens appeared to be covered with very short hairs, were seen (Text-fig. 10g). Although they appear to germinate as readily as the normal prosori, they may, perhaps, sometimes behave as resting spores.

This species of *Micromyces* differs mainly from species already described in having a smooth-walled prosorus. Although the ornamentation of the prosoral wall appears to be so variable in species of *Micromyces*, and may prove an unsuitable character upon which to base specific distinction, it, nevertheless, seems advisable to refer this organism to a new species. *M. laevis* is suggested, taking its name from the constant, smooth-walled prosorus.

#### *Micromyces laevis* n.sp.

Prosorus spherical, 7.6–18  $\mu$  in diameter, with a smooth colourless wall; sorus endobiotic, smooth walled, spherical, with four to eight sporangia; soral wall splitting into as many parts as sporangia, zoospores numerous, 1  $\mu$  in diameter, with a single oil globule, and posterior flagellum. Resting spores, 12.7  $\mu$  in diameter, brown walled, rarely covered with numerous short fine hairs; on germination functioning as a prosorus.

In *Mougeotia* sp., Clay Pond, Wray Castle, Windermere, England.

*Micromyces laevis* sp. nov.

*Prosorus sphaericus*, 7·6–18  $\mu$  diam., tunica laevis, hyalina. Sorus endobioticus, laevis, sphaericus, sporangia 4–8 complectens, tunica in tot partes quot sporangia dissiliente. Zoosporae numerosae, 1  $\mu$  diam., guttula oleosa singula praeditae, postice uniflagellatae. Sporae perdurantes, 12·7  $\mu$  diam., tunica brunnea, rare breviter pilosa, germinatione ut prosoris se gerentes.

Hab. in *Mougeotia* sp., Clay Pond, Wray Castle, Windermere, Angliae.

As mentioned earlier in this paper (p. 69) the older literature contains many records of spiny bodies, asterospheres, the true nature of which was then unknown, causing hypertrophy amongst members of the Conjugales. There is little doubt that some of these bodies are the prosori of species of *Micromycopsis* and *Micromyces*.

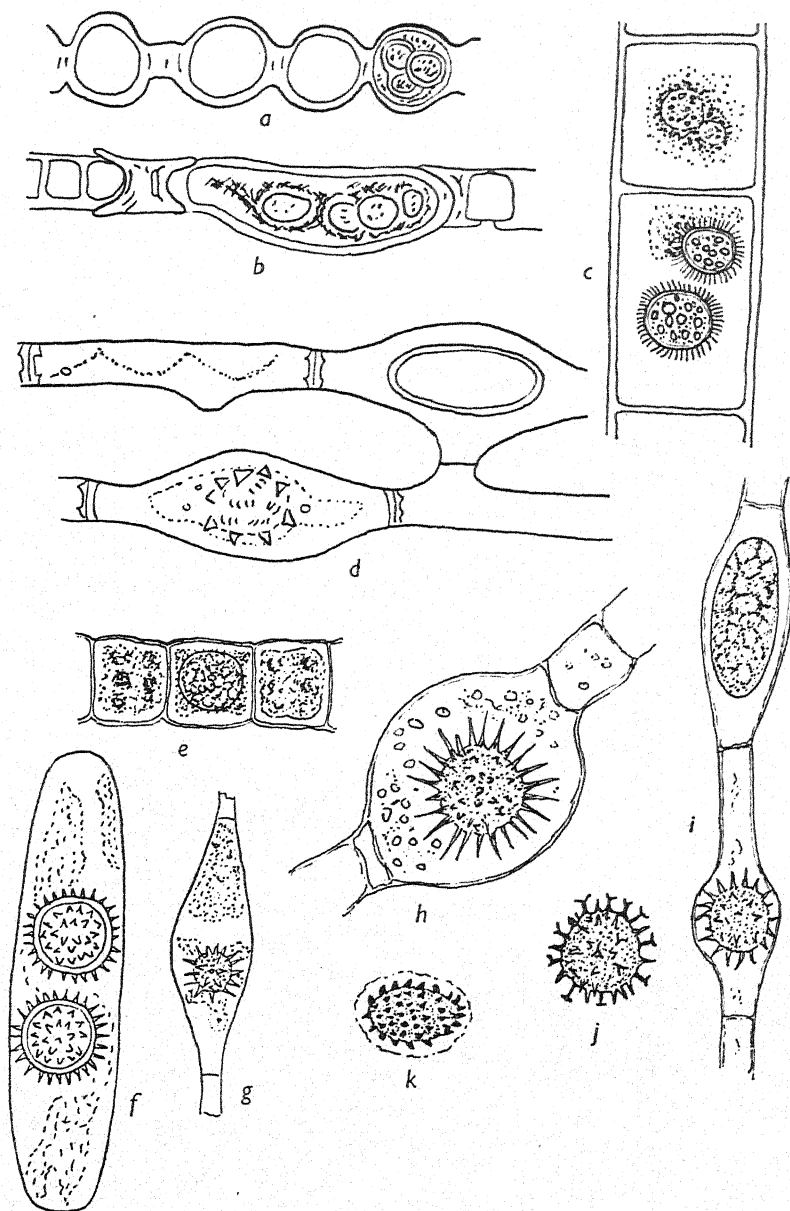
The earliest record is by Thwaites (1846–7), who found globose bodies with several long spines, causing inflation of *Mesocarpus scalaris*, and suggested that they may be an abnormal growth of the nucleus, or an internal parasite. Later, Shadbolt (1852) records spiny bodies in *Zygnema quadratum* (Text-fig. 11i) where sometimes the spines bifurcated (Text-fig. 11j), and in *Zygnema varians* with longer and more acute spines (Text-fig. 11h). Figures and a description are also given of an ellipsoidal body with short spines arranged on it in a regular helix in cells of the blue-green alga *Lyngbya floccosa* (Text-fig. 11k). Further investigations on this form would have been interesting as so far *Micromyces* and *Micromycopsis* spp. are known only as parasites of Conjugales. Other records of spiny bodies are given by Smith (1853), Pringsheim (1895), De Bary (1858) and Reinsch (1875). The parasites figured by Pringsheim (1895) in *Spirogyra* sp. appear to be of protozoan origin.

The genus *Micromyces* was erected by Dangeard in 1889 and now includes five species. Although the type species *M. zygonii* Dangeard has many records, some must remain doubtful as germination of the prosorus has not been observed. Records where germination is known are as follows:

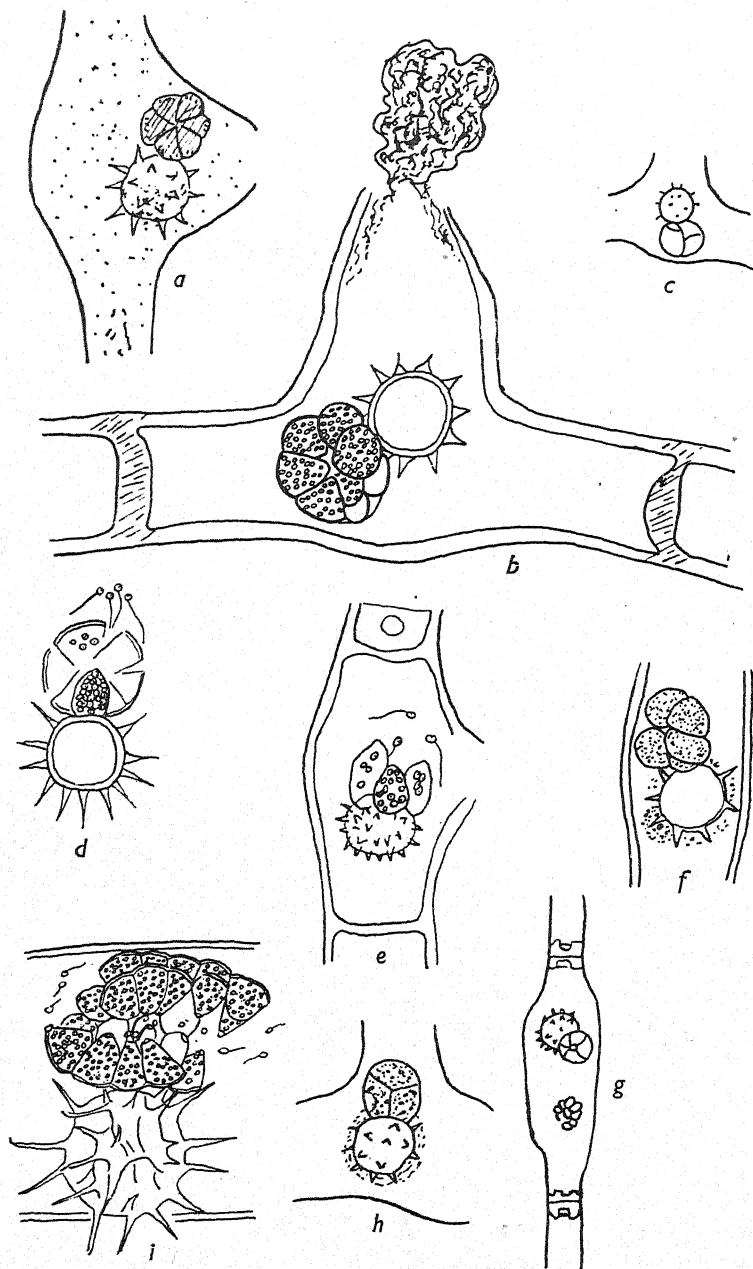
On *Zygonium* sp. Dangeard (1889), *Spirogyra quadrata* Denis (1926), France; *Mougeotia* sp. Petersen (1910), Denmark; *Mesocarpus scalaris* Minden (1915), *Mougeotia scalaris* Heidt (1937), Germany; *Mougeotia* sp. Huber-Pestalozzi (1931), Switzerland; *Mougeotia* sp., *Zygonium* sp. Couch (1937), *Mougeotia* sp. Sparrow (1943), United States.

Smooth-walled prosori causing great swelling of *Zygonium* cells (Text-fig. 11a, b) were found by De Wildeman (1891) and referred to *Micromyces zygonii*. Similar bodies were found by the author in *Zygonium* sp. from Rusland Bog, Lancashire, in July 1946. In neither instance has the germination of the prosorus been observed, and therefore De Wildeman's record still remains in doubt. Other incomplete records are given by Schulz (1922; fig. 91) in *Netrium* sp. (Text-fig. 11f), and also (1923, figs. 10–11: cited from Sparrow, 1943) in *Mougeotia* sp. Again Denis (1926) figures an indeterminate body with a characteristic ornamentation in *Spirogyra tenuissima* (Text-fig. 11d). The surface of the wall is thick, finely and regularly plaited; in addition there are hyaline ridges, more or less sharp, with or without marginal teeth. Other stages are unknown.



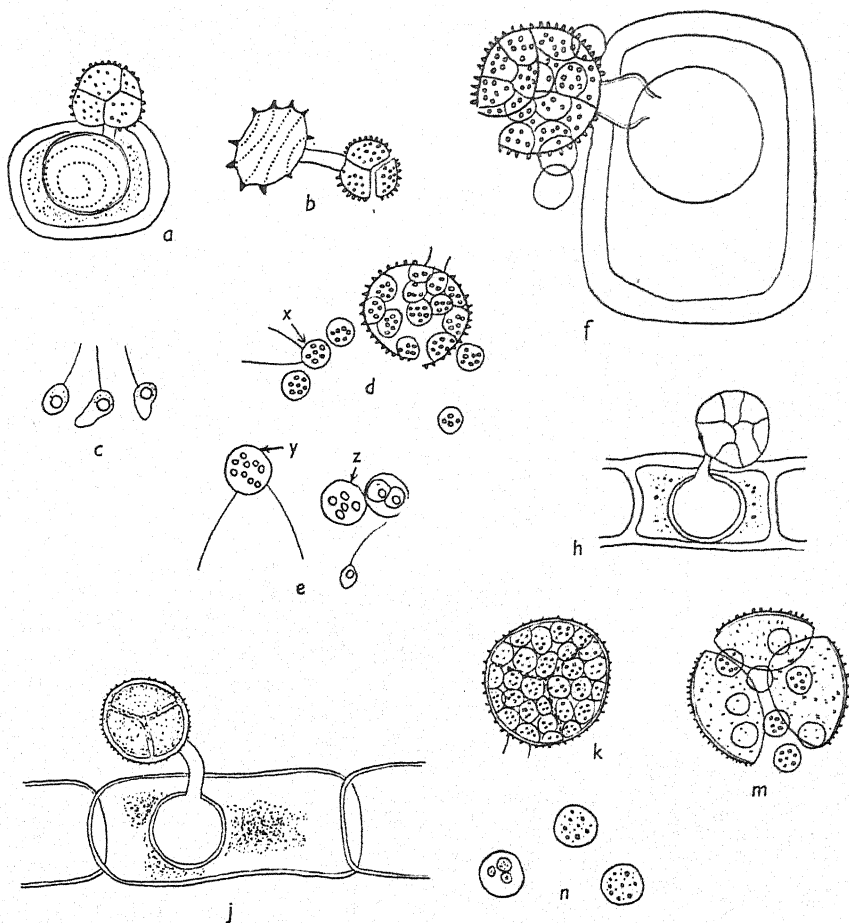


Text-fig. 11. *a, b*, smooth-walled prosori of *Micromyces zygogonii* in *Zygonium* sp. (after de Wildeman, 1891). *c*, two prosori of *Micromyces spirogyrae* in *Spirogyra inflata* (after Skvortzow, 1925). *d*, unidentified internal parasitic body in *Spirogyra tenuissima* (after Denis, 1926). *e*, prosorus of *Micromyces cristata* in *Hyalotheca dissiliens* (after Cejp, 1933). *f*, spiny bodies in *Netrium* sp. (after Schulz, 1922). *g*, asteridia in *Mesocarpus scalaris* (after Smith, 1853). *h*, asteridia in *Zygnema varians*; *i, j*, in *Zygnema quadratum*, *j*, specimen with forked spines. *k*, asteridia in *Lyngbya floccosa* (*h-k* after Shadbolt, 1852).



Text-fig. 12. *a*, *Micromyces zygogonii* Dangeard, germinated prosorus with sorus of sporangia in *Mougeotia* sp. (after Huber-Pestalozzi, 1931). *b*, *Micromyces zygogonii*, prosorus with sorus of sporangia in *Mougeotia* sp. (after Heidt, 1937).  $\times 620$ . *c*, *Micromyces zygogonii* (after Petersen, 1910). *d*, *e*, *M. zygogonii* in *Zygogonium* (after Dangeard 1889). *f*, *M. zygogonii* (after Couch, 1937). *g*, *M. zygogonii* in *Spirogyra quadrata* (after Denis, 1926). *h*, *Micromyces petersenii* Scherffel in *Mougeotia* sp. (after Scherffel 1926). *i*, *Micromyces longispinosus* Couch (after Couch, 1937).

For a form similar to *Micromyces zygonii*, but differing in the larger size of the zoospores,  $6 \times 2 \mu$  rather than  $1 \mu$  diameter, Scherffel (1926) erected the second species of the genus, *M. petersenii*. The only other species which has been adequately studied is *M. longispinosus* Couch (1937) (Text-fig. 12i). *M. spirogyrae* Skvortzow (1925) (Text-fig. 11c) is of uncertain affinities, as neither the germination of the prosorus, nor the structure of the zoospores



Text-fig. 13. *a-f*, *Micromyces cristata*. *a*, empty intramatrix prosorus with spine spirals in polar view and extramatrix sorus.  $\times 460$ . *b*, as (*a*), spine rows of prosorus seen from the side.  $\times 460$ . *c*, non-swarming swimmers.  $\times 680$ . *d*, rounded sporangia of the spherical sorus, which probably arose from the non-swarming swimmers, in part inside, in part outside the sorus membrane; by (*x*) one has developed two fine thread-like processes.  $\times 680$ . *e*, (*y*) sporangium with two processes; (*z*) without thread-like processes; nearby a discharged swimmer. *f*, smooth-walled prosorus with sporangiosorus (after Scherffel, 1926). *h*, *Micromyces fischeri*, smooth-walled intramatrix empty prosorus in *Zygonium*; extramatrix sporangiosorus with a network of empty zoospores.  $\times 680$  (after Scherffel, 1926). *j-n*, *Micromyces zygaemicola*. *j*, sporangiosorus with exit tube and empty initial stage. *k*, sporangiosorus. *m*, sporangiosorus torn into three parts with sporangia escaping. *n*, sporangia, one with three spores (after Cejp, 1932).

have been observed. Lastly, the *M. mesocarpi* of De Wildeman (1900) differs in forming an epibiotic sorus, which suggests that it more closely resembles members of the genus *Micromycopsis* as at present defined. Three species of *Micromycopsis* Scherffel have been described, namely, *M. cristata* and *M. fischeri* (Scherffel, 1926), and *M. zygaemicola* (Cejp, 1932). *M. cristata* (Text-fig. 13a-f), found in *Hyalotheca dubia* from Hungary, has only one other record, by Cejp (1933) from Czechoslovakia, growing on *H. dissiliens* (Text-fig. 11e). However, germination of the prosorus was not observed, and this organism may equally belong to the genus *Micromyces*. A variety *M. cristata* var. *minor* was erected by Sparrow (1932) for a small form bearing sharp instead of blunt spines on the soral wall. The second species, *M. zygaemicola* Cejp, differs from the above in having a smooth rather than spiny prosoral wall, and in the sorus being formed away from the host cell instead of on its surface (Text-fig. 13j). The incompletely known *M. fischeri* Scherffel differs from the other species in its smooth-walled colourless sorus which is divided into sporangia by radially arranged sutures (Text-fig. 13h, and see p. 74).

In view of the further studies on these organisms it has become increasingly difficult to find trustworthy characters on which to base generic and often specific distinction, and it is clear that in the near future *Micromyces* and *Micromycopsis* may have to be merged into one genus. It is also evident that Scherffel's observations (although not continuous) on the rare occurrence of a flagellate sporangial stage in *M. cristata* were probably correct. Such a phase is normally present in *M. fischeri* and *Endodesmidium formosum*. The latter may be regarded as the most primitive type, the primary non-swarming swarmers being formed separately. *Micromycopsis fischeri*, however, differs from *Endodesmidium* in the sorus being divided into sporangia each of which liberates five or more of these primary zoospores, which after liberation behave in a similar manner. Passing to *Micromycopsis cristata* this flagellate sporangial stage is rarely recorded and more typically the behaviour is as in *M. zygaemicola* and *Micromyces* spp., where there is apparently only one sporangial stage liberating the normal chytridiaceous zoospores, which correspond to the secondary zoospores of the former types.

It thus appears that during the course of development of these organisms the primary non-swarming zoospore stage and the subsequent formation of secondary sporangia is suppressed and we get, as in most species of *Synchytrium*, a sorus of sporangia immediately giving rise to chytridiaceous zoospores. *S. fulgens* Schröter may be considered as representing the culmination of suppression in which the sorus of sporangia is formed within the prosorus.

#### SUMMARY

The morphology and life history of seven aquatic Synchytriaceae are described. *Micromyces zygonii* Scherffel, *M. petersenii* Scherffel and *Micromycopsis fischeri* Scherffel are new records for Great Britain; *Micromyces laevis*, *Micromycopsis intermedia* and *M. mirabilis* are new species; and *Endodesmidium formosum* gen. nov. sp. nov.

In *Endodesmidium* the sporangia emerge from the sorus as non-swarming

zoospores, and it is believed this represents a primitive condition within the group.

References to 'asterospheres' in the older British literature are cited, and the validity of records of *Micromycopsis* and *Micromyces* spp. are considered.

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## EXPLANATION OF PLATES

*Endodesmidium formosum* n.gen.

## PLATE VII

- Fig. 1. Healthy cell of *Netrium oblongum* (De Bary) Lütken.  $\times 680$ .  
 Fig. 2. Young parasite in oil globule stage at (a); host contents beginning to degenerate.  $\times 660$ .  
 Fig. 3. Two naked granular prosori.  $\times 640$ .  
 Fig. 4. Stained mature prosorus; (b), more densely staining area, possibly the nucleus. (c) vacuole once occupied by the oil globule.  $\times 600$ .  
 Fig. 5. Walled prosorus in *Cylindrocystis*, (d), anterior conspicuous oil globule.  $\times 840$ .  
 Fig. 6. Naked prosorus in *Cylindrocystis*.  $\times 650$ .  
 Fig. 7. Germinated prosorus with almost mature sorus in *Netrium*.  $\times 700$ .  
 Fig. 8. Dehiscent sorus; a few sporangia have failed to escape.  $\times 660$ .

## PLATE VIII

- Fig. 1. A *Netrium* cell containing three empty prosori of *Endodesmidium formosum* n.sp. with their respective sori; the latter are out of focus. The sporangia are clearly visible and that at (x) has two oil globules delimited, each indicating the position of a zoospore.  $\times 1150$ .

PLATE IX. *Micromycopsis fischeri* Scherffel

- Fig. 1. Naked prosorus in *Tetmemorus Brebissonii*; the host content is little disorganized.  $\times 860$ .  
 Fig. 2. Mature prosori.  $\times 840$ .  
 Fig. 3. Early stage in development of the sorus.  $\times 830$ .

## PLATE X

- Fig. 1. *Micromyces zygonii* Dangeard, naked prosorus in *Spirogyra* sp.; note absence of swelling of the host cell.  $\times 880$ .  
 Fig. 2. *M. zygonii*, germinated spiny prosorus with dehiscent sorus; one sporangium still retains its zoospores.  $\times 860$ .  
 Fig. 3. *M. zygonii*, brown-walled resting spore.  $\times 800$ .  
 Fig. 4. *M. petersenii* Scherffel, empty prosorus with a few long spines in *Mougeotia* sp.  $\times 600$ .  
 Fig. 5. *M. petersenii*, young naked prosorus.  $\times 650$ .  
 Fig. 6. *M. laevis* n.sp., two young prosori in *Mougeotia* sp.  $\times 630$ .  
 Fig. 7. *M. laevis*, mature prosorus.  $\times 570$ .  
 Fig. 8. *M. laevis*, almost mature prosorus with a halo of host globules.  $\times 600$ .  
 Fig. 9. *Micromycopsis intermedia* n.sp., sorus with five dehiscent sporangia viewed from above.  $\times 740$ .  
 Fig. 10. *M. intermedia*, two prosori in *Zygnema* sp.; one has formed an exit tube and extramatrical sorus.  $\times 920$ .

## PLATE XI

- Fig. 1. *Micromycopsis mirabilis*, part of a *Closterium* cell with eight prosori.  $\times 430$ .  
 Fig. 2. Immature prosorus with developing spines.  $\times 650$ .  
 Fig. 3. Empty prosorus with sorus of sporangia.  $\times 430$ .  
 Fig. 4. Unidentified *Micromycopsis* sp.  $\times 705$ .

(Accepted for publication 28 August 1947)

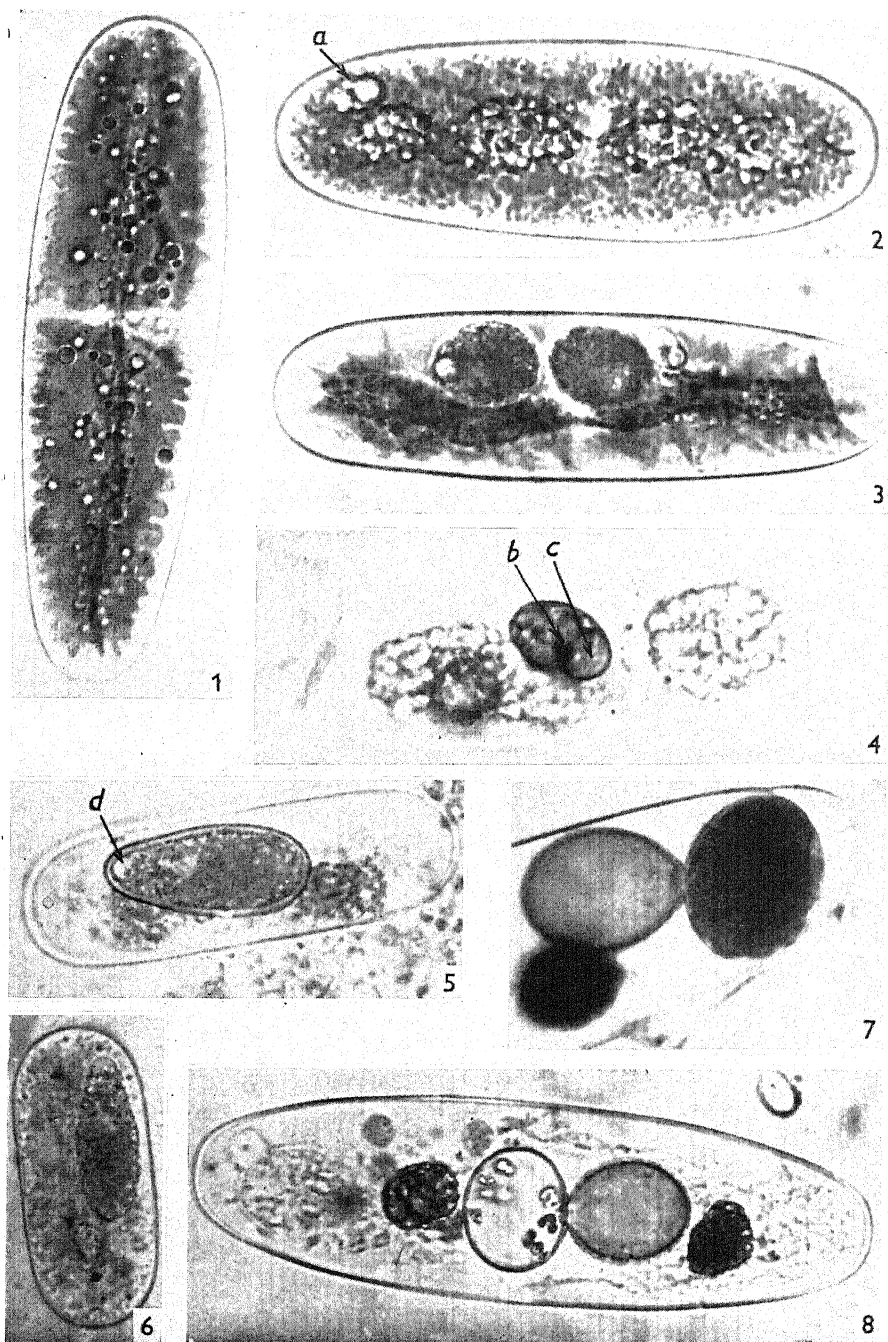


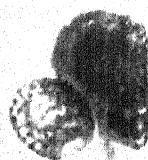
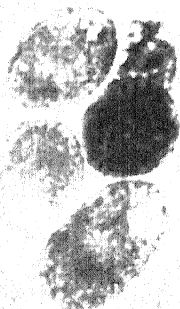




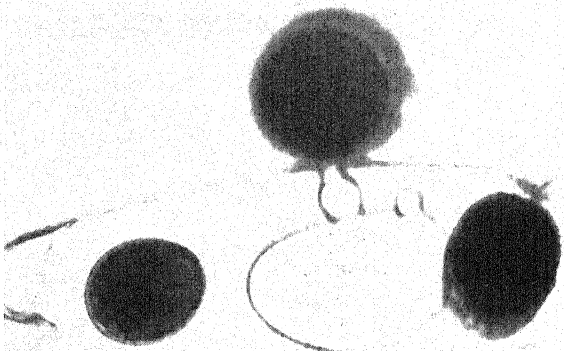
Fig. 1



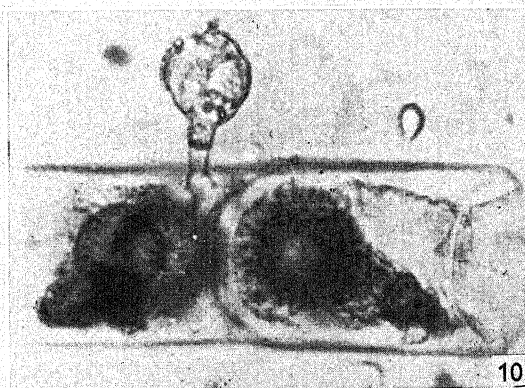
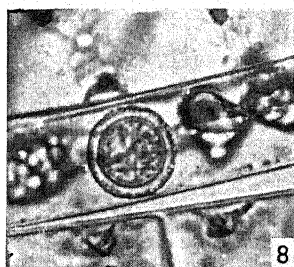
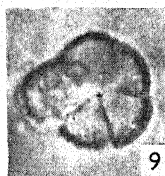
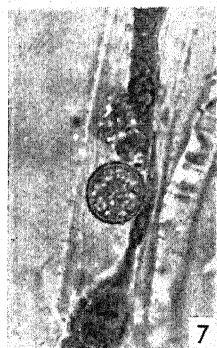
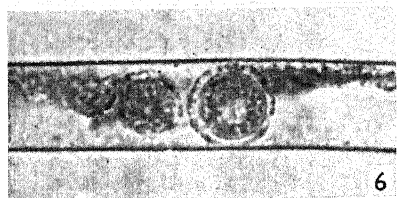
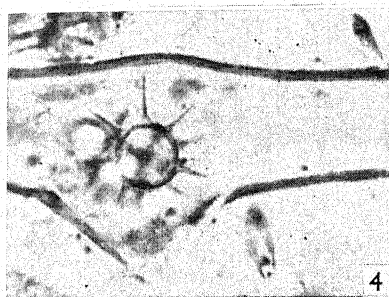
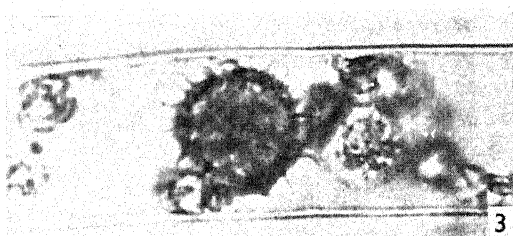
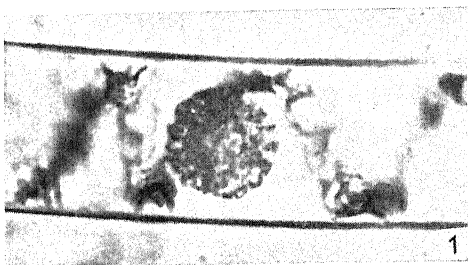
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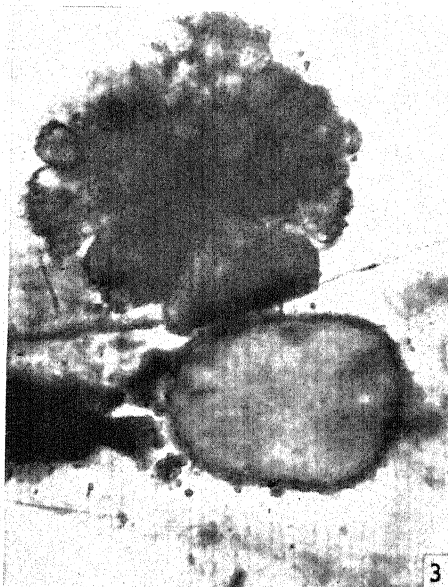
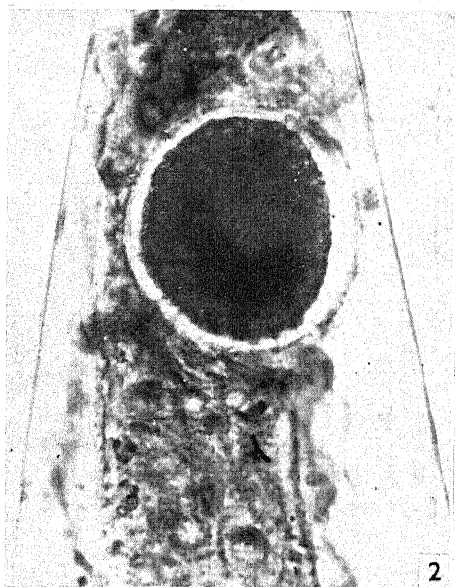
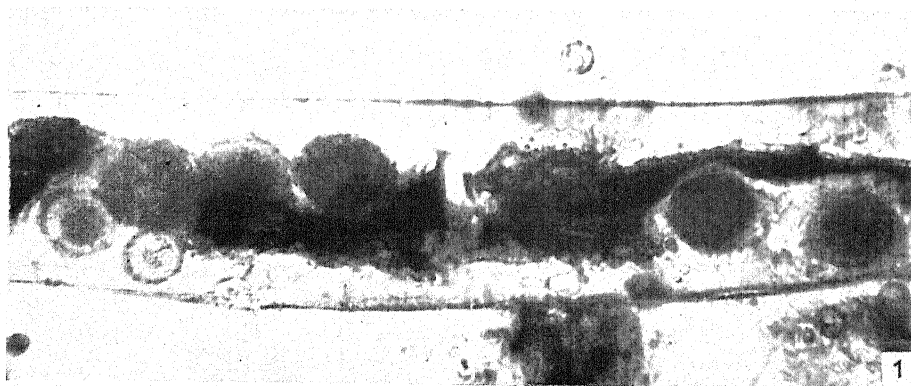


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## NEW AND INTERESTING PLANT DISEASES

By W. C. MOORE, *Plant Pathology Laboratory, Harpenden, Herts*

33. LEAF BLOTCH OF *GEUM* (*ZYTHIA FRAGARIAE* LAIBACH)

Early in September 1941 I noticed that a number of plants of *Geum*  $\times$  *Borisii*, growing in a nursery bed at Harpenden, showed dark olive-brown blotches on the leaves. The blotches were visible on both sides of the leaves, irregular in shape, scattered or spreading in from the margins in V-shaped fashion, and they occasionally covered the whole leaf. Numerous pycnidia were present on the blotches: they were mainly epiphyllous, occasionally hypophyllous, immersed then erumpent, subglobose, pale brown, thin-walled,  $130\text{--}190\mu$  ( $\sim 300\mu$ ) in diameter. The pycnospores were  $5\text{--}7 \times 1\cdot5\text{--}3\mu$ , hyaline, short rod-shaped, with rounded ends and a prominent guttule at each end.

The fungus was tentatively identified as *Phyllosticta gei* Bres., which has been listed by Grove (*Coelomycetes*, i, 39) on *Geum urbanum* and *G. rivale* in Ayrshire. I have examined a slide, prepared from type material of this species (on *G. urbanum*, Königstein, 29 May 1897) kindly sent to me by Miss E. M. Wakefield, and the Harpenden material appears to me to be identical with it. The fungus, however, is not a good *Phyllosticta*: it is a species of *Zythia*, and I can see no difference between it and *Z. fragariae* Laibach, which has been found on strawberry leaves in several parts of England and Wales (Moore, 1943; Wormald, 1944). Moreover, Dr Wormald has told me that he succeeded in infecting leaves of *Geum* with *Zythia* from strawberry.

*Phyllosticta gei* Bres. (*Hedwigia*, 1900, 325) is antedated by *P. gei* Thüm. (*Bull. Soc. Imp. Nat. Moscou*, LVI, Part 2, 1881, 130) which, judged from its description, is a different fungus, with pycnidia 'opaco-nigricis', and therefore probably a true *Phyllosticta*. It would seem that two species are passing current under the name *P. gei*, and confirmation of this was given me in a letter received from Dr I. Jørstad, who informed me that the *Phyllosticta* commonly occurring in Norway on *Geum rivale* and *G. urbanum* does not fit *Phyllosticta gei* Bres. but is clearly identical with Jaap's fungus on *Geum* referred to by Diedicke in *Krypt. fl. Mark Brandenb.* ix, Pilz, vii, 55 (1912). As *P. gei* Bres. is a homonym and therefore invalid, *Zythia fragariae* Laibach is a valid name for the fungus that causes leaf blotch of *Geum* and strawberry. Its perfect state, according to Wormald (1944), is a species of *Gnomonia*, possibly identical with *G. herbicola* A. L. Smith (1910).

34. DOWNY MILDEW ON *ALYSSUM SAXATILE* (*PERONOSPORA*  
*GALLIGENA* BLUMER)

On 11 November 1946 Mr W. Buddin sent me some leaves and young seedlings of *Alyssum saxatile* affected with a Downy Mildew. They had been sent to him from a nursery at Slough (Bucks), and according to particulars which he later obtained, the affected plants had been raised from seed



sown rather thickly in shaded beds under glass in May. The mildew was first noticed, probably in July, and dusting with sulphur failed to check it. Though previously thinned, the seedlings were rather leggy when transplanting was carried out on 7 November.

The affected leaves showed what appeared to be small yellowish or brownish green galls or blisters, about two or three millimetres across and sometimes as high, on the upper surface. Actually, however, these small areas were 'humps' on the upper surface where the leaf tissues were locally puckered, and corresponding 'hollows' were seen on the lower surface when the leaf was turned over. Occasionally the humps appeared on the lower surface and the midribs below were locally swollen. The leaves had a yellowish cast and a number of them were deformed, especially when the midrib was swollen. Conidiophores of a *Peronospora* were present on both sides of the leaves, but were particularly abundant on the underside. For the most part they developed in small tufts on or just around the puckered spots, and the hollows on the underside were usually filled with a white mass of mycelium and conidiophores. There were, however, isolated conidiophores, or tufts of them, on those parts of the leaves which showed no obvious sign of puckering, and especially on the under surface. The conidiophores were up to 1.2 mm. long, rather lax, unseptate, 9–12  $\mu$  broad, and unbranched except at the distal end where there were a few main branches that divided repeatedly to give numerous ultimate branchlets, tapering to a width of 2  $\mu$  and ending in curved prongs with rounded tips, each bearing a conidium. The conidia were mostly broadly egg-shaped, occasionally spherical, 15–24  $\times$  14–19  $\mu$  (average of twenty-five conidia, 18  $\times$  15  $\mu$ ), with thin smooth walls and granular contents.

The fungus was clearly identical with *Peronospora galligena* Blumer, which Blumer (1938) described as common around Berne in Switzerland on *Alyssum saxatile*, and as occurring in the Botanic Gardens at Berne on its varieties *citrinum* and *compactum*. The same species was reported on *A. saxatile* soon afterwards in various parts of Germany, including the Rhineland (Laubert, 1938) and the Dresden area (Pohlig, 1939), as well as on var. *citrinum* at Kiel (Pape, 1938).

Gäumann (1918, 1923) had previously recognized two subspecies of *Peronospora parasitica* (Fr.) Tul. within the host genus *Alyssum*, namely *Peronospora alyssi calycini* Gäum. on *Alyssum calycinum* and *Peronospora alyssi incani* Gäum. on *Alyssum incanum*. The conidia of *Peronospora alyssi calycini*, averaging 25  $\times$  19  $\mu$ , are much larger than those of *P. galligena*, but according to Blumer the last-named species is not widely different morphologically from *P. alyssi incani*, or from *P. berteroeae* Gäum on *Berteroa* (*Alyssum*) *incanum* and *Peronospora camelinae* Gäum. on *Camelina microcarpa*. Nevertheless, in inoculation experiments with *Peronospora galligena*, Blumer could infect only *Alyssum saxatile* and its varieties *citrinum* and *compactum*. He failed to infect twenty other crucifers, including *A. calycinum*, *A. incanum* and *Camelina microcarpa*, and therefore regarded his fungus as a distinct species. He also considered it to be distinct from a Downy Mildew found on the native *Alyssum saxatile* in Moravia and named by Hruby (1930), without description, *Peronospora alyssi arduini*. As a specialized form of the very common *P. parasitica* the fungus occurring on *Alyssum* in this country may well be widely distributed,



though unnoticed. Over fifty years ago Halsted (1891) reported *Peronospora parasitica* in New Jersey on greenhouse plants of *Alyssum maritimum* growing near affected radishes.

### 35. DOWNY MILDEW OF THE VINE (*PLASMOPARA VITICOLA*)

The Downy Mildew of the vine appears still to be quite rare in Great Britain, and some interest therefore attaches to new records of it. W. G. Smith (1886) was able to write of this mildew: 'at present, although expected every season, it has not yet been recorded from Britain', but within a few years M. C. C[ooke] (1894) reported that two sets of infected leaves had been received from places in Britain more than 100 miles apart—and from specimens in Herb. Kew and in Herb. Mus. Brit. it is certain that one of these places was Derby. During the next thirty years *Plasmopara viticola* (Berk. & Curt. ex de Bary) Berl. & de Toni was recorded a few times in different parts of the country, but little reliance can be placed on these records and one or two of them proved on closer inquiry to have been errors for *Uncinula necator* (Schw.) Burr., the true vine mildew. The third authentic record of the *Plasmopara* was not made until Harrison and Ware (1926) found it on an outdoor vine in a private garden at Wye, Kent. The infected vine was cut off at ground level at the time and the above-ground parts were burnt. The rootstock, which was left intact, grew vigorously during the next few years, and in September 1932 Downy Mildew was found in small quantity on the new growth. About the same time it was also collected on the ornamental *Vitis coignetiae* Pulliat in a garden at Haslemere, Surrey. The sixth known occurrence in Britain was in 1936 on a very old outdoor vine in a garden at Wye about 300 yards from the earlier infected vine, which had been pulled up and completely destroyed after the 1932 outbreak. In 1941 a substantial attack was observed on a Miller's Burgundy vine growing in the open at Wye on a house wall about a quarter of a mile from the earlier infected vines: though three plants, Brant, Chasselas rose (Reine Olga) and Early Saumur Frontignan, growing on the walls of the same house were not attacked. The eighth and most recent\* record was made in August 1947, when Mr F. G. Ordish sent me mildewed leaves taken from a fairly severely affected Teinturier Grape, *Vitis vinifera* L. var. *purpurea*, growing in his garden at Yalding, Kent.

One of the first two outbreaks, and perhaps both of them, occurred under glass, for Cooke (1894) wrote that at one locality the vine attacked 'was a different variety from others which the house contained', but all the more recent records have been on outdoor plants. Early fears that *Plasmopara viticola*, once introduced, would rapidly become established in Britain have not been justified, and there is now no reason to suppose that it will ever become a menace here.

### 36. PRE-STORAGE BLACK HEART IN POTATO TUBERS

Black Heart is not uncommon in clamped potatoes and in tubers stored in badly ventilated places, and in the United States of America it has at

\* A fairly severe attack occurred on a number of vines at Oxted, Surrey, in October 1948.

times caused considerable loss among potatoes being transported in stove-heated railway wagons. Indeed, it is apt to occur whenever potato tubers are subjected to temperatures of 38–48° C. for at least fifteen to twenty hours, or at much lower temperatures if ventilation is poor and the supply of oxygen sufficiently low. Under such conditions the central part of the tuber is killed, and the dead part either becomes uniformly dark grey to purple or inky black in colour, or exhibits simple or intricate patterns on a somewhat lighter background. Sometimes the blackened tissues contract, giving rise to a smaller or larger hollow in the centre, with a black lining. In this state the defect may easily be confused with true Hollow Heart, a seasonal non-parasitic trouble observed chiefly in wet summers or when a drought in early summer is followed by rain in August or September. In Hollow Heart it is rare for the cavity to show a black lining.

Observation has made it clear that Black Heart is not invariably a storage disease, but one that under special conditions may occur immediately after digging or even while the tubers are still in the ground. Temperature is a controlling factor, though there is obviously a close relation between the development of Black Heart, temperature, time of exposure, and oxygen supply. The first time I saw Black Heart in potatoes at digging time was during the late summer of 1933—a year that saw the hottest and driest August in England and Wales for at least thirty years. Specimens began to arrive in the first few days of September from gardens in the Bristol area, in Surrey and in other southern districts. In some tubers the whole of the central part was blackened, while in others there was only a small more or less central cavity surrounded by a brown or black lining. The same symptoms were also found to be fairly common in mid-Wales at lifting time, but it was not always clear, either there or elsewhere, whether the symptoms were present before lifting or whether they first developed a few days later, possibly after the potatoes had been left lying on the ground to dry in the sun. On one farm near Aberystwyth, however, the symptoms were known to have been present before digging. They were found in May Queen grown in light loam overlying shale, on a field with a southern slope of one in eight: while King Edward and Kerr's Pink growing alongside were unaffected. Other varieties affected that year were Sharpe's Express, King George and Up-to-Date. I did not see this pre-storage form of Black Heart again until 1938, when one sample only was received from Hailsham, Sussex. The variety was King George, grown in light soil: the affected tubers had been dug on 30 July, exposed to the sun over the weekend, and were found to have black centres on 2 August. Further complaints about this form of Black Heart were received in August 1947, during a month that for many parts of Britain was the sunniest and driest on record. From 19 to 28 August, specimens were received from Dorset, Sussex, Essex and the London area. Some of the tubers had been exposed to hot sun for two or three days after digging, but there was clear evidence in at least one instance that the tubers were affected 'as lifted'.

These observations strongly suggest that Black Heart may develop in potato tubers before or immediately after digging in seasons when temperatures in August are unduly high. As the defect cannot normally be

detected until the tubers are cut, it is unwise to assume without more ado, when Black Heart is found during the winter months in clamped or stored tubers, either that the clamping conditions are unsatisfactory or that ventilation in the storage chamber is faulty. The true explanation may be that the symptoms were induced months before as an effect of high soil temperatures or of hot sun while the tubers were still attached to the parent plant.

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(Accepted for publication 4 September 1947)

## THE TEACHING OF MYCOLOGY

[A Report prepared by a Committee, appointed by the Council in April 1945, consisting of G. C. Ainsworth, Miss E. M. Blackwell, F. T. Brooks, C. G. C. Chesters, Miss L. Hawker, C. T. Ingold (*Chairman*), J. Ramsbottom, G. Smith, W. H. Wilkins and S. P. Wiltshire. The Report was circulated privately in June 1946.]

### FOREWORD

The dearth of trained mycologists has been apparent for many years and had serious effects during the war period.

In 1944 the Council of the British Mycological Society issued a report on 'The need for encouraging the study of systematic mycology in England and Wales', drawn up by a specially appointed Committee. Constantly in its deliberations the problem of training arose, and, after some delay, the Council appointed a further Committee, composed mainly of those with practical experience, to report on 'The Teaching of Mycology'.

This Report was approved by the Council and it was decided to print and circulate it but to defer publication. J. RAMSBOTTOM (*President*).

### I. MYCOLOGY DEFINED

Mycology is the study of all aspects of Fungi—their morphology, physiology, ecology, cytology, genetics, and taxonomy. Fungi form a huge group of probably some 100,000 species and their distinctive form and nutrition give to their study a special interest heightened by their enormous economic importance. Fungi are of great significance in industry and in medicine, but they are especially important as the principal causal organisms of plant disease. The great development of Plant Pathology during the past century has led some to the view that Mycology and Plant Pathology are synonymous, but this attitude is quite unjustifiable. Fungi should be studied primarily for their own sake, although, in a balanced course on Mycology, the economic aspects must receive their due emphasis.

### II. MYCOLOGY IN RELATION TO BOTANY

A good case can be made out for considering Fungi as a kingdom of organisms co-equal with Plants and Animals. However, the study of Fungi has developed, in the main, within the confines of Botany and on grounds of tradition, and also because of the paramount importance of Fungi in Plant Pathology, it is generally considered desirable that they should continue to be studied as part of Botany. Only a few of those who lecture on Fungi in the Universities are in favour of Mycology being taught as a separate subject like Bacteriology.

In an Honours course in Botany the amount of time given to the study of Fungi varies from one-tenth to one-sixth of the whole course. The Committee agrees that the course leading to a first degree in pure Science (as in Arts) should primarily be cultural rather than technical and that it, therefore, is not desirable in a Botany degree to emphasize one aspect of

the subject at the expense of others. Nevertheless, they feel that Fungi, if properly treated, certainly deserve one-sixth of the available time.

It is suggested that for students taking an Honours degree in Chemistry, Mycology might form a more valuable subsidiary than Botany or Biology. If a course of this type were developed in any of the Universities, it would be most desirable that it should be a general course in Mycology and not a technical course in Industrial Mycology. The Committee regard it as a basic principle that the study of Fungi as a branch of pure science should always precede a study of Applied Mycology.

It should be noted that the teaching of Mycology in the Universities is not confined to the departments of Botany. Students studying for degrees in Agriculture, especially for Honours degrees in Agricultural Botany, follow special courses usually conducted by lecturers of the Faculty of Agriculture. Again in at least one Department of Biochemistry, a School of Industrial Mycology is developing. The fact that Mycology is no longer the monopoly of Botany should have a stimulating effect on the development of the subject.

Most departments of Botany have a lecturer on the staff with special qualifications in Mycology. It is only in the smaller departments that this is not so. This involves no criticism of these small departments, which obviously cannot have a specialist in all the important branches of Botany.

Many departments have special mycological laboratories, but others with cramped quarters have not, and cannot expect to have such facilities. However, it is essential that all Botany departments should be fully equipped for the special discipline of mycological work, including adequate library facilities. Liberal supplies of apparatus are necessary if all post-intermediate students are to have the opportunity of studying Fungi in the laboratory as living organisms and of carrying out experiments in pure culture.

### III. THE CONTENT OF MYCOLOGY

Fungi have suffered in the past from their relegation to a subphylum of the Thallophyta with the implication that they are degenerate Algae. Like Algae, Fungi have tended to be studied from the standpoint of evolutionary morphology with over-much emphasis on sexuality and cytology. The 'type system' has largely dominated their treatment at the intermediate and subsidiary levels.

The Committee suggests that in the study of Fungi their consideration as living organisms is of special importance. Botany is primarily an observational science, but an increasing element of experimental work is desirable. The technique of isolating and growing Fungi in pure culture, and the study of their physiological reactions under these conditions is simple and interesting, and is not only of great value from the standpoint of Mycology, but also from the point of view of the experimental method in Botany. However, it is undesirable that Fungi should be treated merely as units in a collection of type cultures. Where possible, some species studied by the student should be isolated by him from natural substrata.

A study of Fungi as living organisms involves not only an extension of culture work, but also an extension of field work. Individual practical

work by Honours students on particular systematic or ecological groups of Fungi is of special value in promoting the spirit of modern Mycology. An Honours student might well undertake a special study during his course. He might study the bark-inhabiting Fungi of a tree, or the rusts and smuts occurring in his neighbourhood, or investigate the succession of species which develops on horse dung, or study the distribution of Fungi in a wood, or make observations on the watermoulds of a stream, and so on. A student, in this way, by first-hand contact with Fungi, will gain an insight into Mycology which can be obtained in no other way. He will not only learn to know Fungi in nature, but will also become acquainted with some mycological literature and with its use in the identification of genera and species.

Although in a B.Sc. course Fungi should be studied primarily for their own sake, the fundamental facts of their importance, both beneficial and destructive, in Agriculture, Forestry, Industry, and Medicine, should receive more emphasis.

Bacteriology has evolved into a separate science, but bacteria, because of their fundamental physiological similarity, should find a place in a course on Fungi. Their practical study should be more than a mere examination of a few types under the microscope and should include: (1) the method of identification based on physiological tests; (2) the estimation of bacterial numbers (e.g. in soil); and (3) the chief staining methods.

#### IV. POST-GRADUATE WORK

The Committee has no comment to make on the conduct of mycological research. The development of this in the Universities should continue to be free and unfettered.

It may be noted, however, that the volume of mycological research in this country is small and, proportionately, compares unfavourably with that in America. Last century France and Germany led the world in the study of Fungi. The lead has now passed to America. If this country is to play its proper part in the future development of Mycology, much larger sums must be available for research, both fundamental and applied.

The Committee suggests that it is desirable to develop post-graduate courses in Mycology. Although in certain University departments Mycology in the Honours degree receives very considerable emphasis, the Honours graduate in Botany cannot normally be regarded as one sufficiently trained in Mycology to be able at once to fill a mycological post or to engage in mycological research.

Certain post-graduate courses already exist, but there does seem to be a real need to develop further advanced teaching in Mycology as distinct from Plant Pathology, and it is suggested that, in those Universities where the M.Sc. degree may be awarded on examination, an M.Sc. (Mycology) would be a most desirable degree to institute.

In a post-graduate teaching course in Mycology the discipline of the subject and all its aspects should be treated without too much emphasis on any one of them. Such a course would, it is believed, be very suitable for a student who subsequently or concurrently is engaged in the study of Plant Pathology.

It is clear that not all University centres will wish to develop post-graduate courses in Mycology, but it is desirable that a number of such centres of higher mycological teaching should exist.

If a number of post-graduate teaching courses are instituted in the Universities, the character of each will naturally depend on the special branch of Mycology in which the head of the department is most interested. It is, therefore, necessary to have a scheme of co-operation amongst the Universities so that a post-graduate student may be able to select the department in which he wishes to study in accordance with his own special interests, and so that a suitable graduate from a University, not offering a post-graduate training in Mycology, may freely pass to a centre offering such training and be eligible for the degree or diploma at the end of the course.



## THE NEED FOR ENCOURAGING THE STUDY OF SYSTEMATIC MYCOLOGY IN ENGLAND AND WALES

[A Report prepared by a Committee, appointed by the Council in October, 1942, consisting of G. C. Ainsworth, Miss E. M. Blackwell, K. St G. Cartwright, R. V. Harris, W. C. Moore (*Chairman*), J. Ramsbottom, G. Smith, and Miss E. M. Wakefield (*Convenor*). The Report was circulated privately in July 1944.]

### FOREWORD

A critical position in the study of the systematics of fungi in this country has been reached due to the shortage of experienced taxonomists and the absence of facilities for training in taxonomy and nomenclature. The urgency of this problem being apparent to the British Mycological Society, the Council appointed a Committee to study the question and draw up a Report. The Report presented was warmly approved by the Council who decided to circulate it forthwith to all Government Departments, Universities, Institutions and Associations concerned with the activities of fungi. It was decided that the Report should be printed, for convenience, but that it should not be published then, either as a whole or in part. R. W. MARSH (*President*).

### INTRODUCTION

The study of the systematics of fungi has been seriously neglected in this country. During the past thirty years increasing emphasis has been placed on the economic aspects of plant pathology, but there has been a lack of appreciation of the importance of taxonomic mycology to the plant pathologist. Yet increasing importance has been attributed to the damage caused by fungi not only in agriculture and forestry, but to man and animals, in textile industries and in stored products. Their significance in soil fertility, in fermentation industries, and in the production of therapeutic substances is gaining wider and wider recognition. The degree courses in botany at the universities, as they are at present arranged, do not permit adequate training in the taxonomy and nomenclature of fungi. This, added to the fact that the number of experienced taxonomists is diminishing, and few, if any, are being trained to replace them, makes the position critical.

The following Report has been prepared to draw attention to the urgent need for action. In it the history of mycology in this country is traced and a brief account is given of the present position of systematic mycology both at home and abroad. This is followed by a reasoned statement of the necessity for stimulating the study of the subject at the universities, in the central herbaria and in laboratories dealing with economic mycological problems. Certain specific recommendations are made.

## HISTORY AND PRESENT POSITION OF MYCOLOGY IN ENGLAND AND WALES

The comprehensive study of mycology in this country may be considered to have begun with Miles Joseph Berkeley, a clergyman who took up the study of fungi as a hobby. He published his first mycological work in 1836, and from then until his death in 1889 he was responsible for numerous papers and books dealing with fungi collected in all parts of the world. Besides adding greatly to our knowledge of species and genera of fungi and of their distribution, Berkeley recognized that fungi are the cause of decay and disease and so laid the foundations of the science of plant pathology in this country. Following him M. C. Cooke and G. Massee began as amateurs, but eventually obtained official positions at Kew. Worthington G. Smith was a botanical artist who became interested in mycology and worked at the British Museum; C. B. Plowright, who became an authority on the rust and smut fungi, was a medical man. Thus, most of the important early work in mycology in this country was done by men who took it up in their spare time, and distinguished 'amateurs' have continued this valuable work to the present day, though in decreasing numbers. For very many years the terms mycology and plant pathology were regarded as practically synonymous, and such paid posts as existed arose from the need of a 'curator' for national collections, or from a demand for advisory or research work on plant diseases. The only Government posts for mycologists were originally at the British Museum and Kew.

*British Museum.* In 1859 W. Carruthers was appointed Assistant in the Department of Botany, then at Bloomsbury, and in 1871 he became Keeper. He worked mainly with cryptogams and fossil plants but when, in 1871, he also took over the post of Adviser in Botany to the Royal Agricultural Society he was compelled to study plant diseases—work in which he was later assisted by Miss A. Lorrain Smith. G. R. M. Murray, an Assistant in the Department, had charge of fungi and algae from 1876. When he became Keeper in 1895, V. H. Blackman was appointed Assistant in charge of fungi, and was followed (1907–9) by W. E. St J. Brooks. In 1893 Miss Lorrain Smith was employed by the Trustees in arranging the stands illustrating microfungi in the Botanical Gallery and she also took over practically all the identifications of microfungi. By 1910, however, her time had become fully occupied with lichens. J. Ramsbottom (Keeper of Botany since 1930) was appointed Assistant in charge of fungi in 1910. In 1929 Miss F. L. Stephens was engaged to assist in laboratory and routine work, and she was given a permanent appointment in 1936 with the title of Scientific Assistant. Thus, the mycological work in the Department is now carried out by the Keeper (who is much occupied with heavy administrative duties) and a whole-time Scientific Assistant. Since 1930 there have been additional appointments in the Cryptogamic Herbarium, but the full scheme of development with the consequent increase in the number of mycologists has not yet been implemented.

*Royal Botanic Gardens, Kew.* In 1880 M. C. Cooke was appointed to take charge of the lower cryptogams in the Herbarium at Kew, and between 1880 and 1892 he completely rearranged the fungi there and incorporated many large collections, including his own, while at the same time he

published many papers and books on fungi. G. Massee worked with him for some years in a voluntary capacity, and when Cooke retired succeeded him in charge of the lower cryptogams in 1893. From then, until his retirement in 1915, Massee was responsible for both systematic work on fungi and advisory work on plant diseases, not only for the Ministry of Agriculture but also for the Empire. In 1904 A. D. Cotton was appointed as a second Assistant in the Cryptogamic Department, and worked especially with lichens and algae, though he also carried out important mycological investigations. In 1910 Miss E. M. Wakefield was appointed to assist Massee. In 1915, not long after Massee's retirement, Cotton was transferred to the post of Mycologist (Plant Pathologist) in the recently founded Plant Pathology Laboratory of the Ministry of Agriculture and the Kew Herbarium was thus once more reduced to one Assistant in charge of all the lower cryptogams. After the first world war some intermittent help in mycology was obtained from temporary technical assistants, who required much of the Assistant's time to train them and who usually left for more remunerative posts as soon as they had become useful. At present there is still only one mycologist on the staff.

*Imperial Mycological Institute.* In 1921 a further development took place when the Imperial Bureau of Mycology (now the Imperial Institute) was established under the direction of Dr E. J. Butler to deal primarily with problems of mycology and plant diseases in the overseas parts of the Empire. Here at least the increasing need for systematic work on fungi has been acted upon, but of the five mycologists on the staff only two are sufficiently free from routine and advisory work to be able to devote time to any special systematic problems which arise.

In addition to those referred to above there are a few academic and amateur mycologists who have specialized in certain groups of fungi.

#### THE POSITION OF SYSTEMATIC MYCOLOGY ABROAD

It is relevant at this stage to refer briefly to the position of systematic mycology in other countries.

*Europe.* Generally speaking, the position throughout Europe has been much the same as in this country as regards professional posts in systematic mycology. Nevertheless, considerable research work in the subject has been carried out on the Continent by individual pharmacists, school teachers and others who have had training in botany or have become interested in mycology, and by plant pathologists and members of university staffs who work at systematic problems in their spare time. Special mention may be made of Holland, for there a flourishing mycological school has grown up in connexion with the University of Utrecht and the Willie Commelin Scholten Laboratory and Central Bureau for Fungus Cultures at Baarn. It is therefore not without significance that mycologists trained in Holland have done much valuable work in the Dutch colonies, notably Java. Reference may be made also to the Carlsberg Laboratory at Copenhagen, which has been active in the taxonomic study of alcoholic fermentation organisms.

*United States of America.* On the other side of the Atlantic the position is very different. Mycology is taught at most universities, and because

posts in systematic mycology are available, post-graduate students take up the study of special genera or groups of species, with the result that valuable work is being published. Many Agricultural Experiment Stations and State Agricultural Colleges have mycologists on their staffs who carry out research on special groups of fungi, as, for instance, the work on rusts at Purdue University Agricultural Experiment Station. The United States Department of Agriculture, in connexion with the Bureau of Plant Industry, maintains large mycological collections, with one Senior Mycologist in charge and three Assistant Mycologists. There are also large herbaria at various universities and other institutions. In fact great and active interest is widely taken in fundamental problems relating to the taxonomy and nomenclature of fungi, as evidence of which it may be mentioned that the important American Phytopathological Society recently appointed special committees, one for the consideration of fungus nomenclature and the other for the publication of monographs.

*British Empire.* Valuable critical work on fungi is now being carried out in Australia, New Zealand, Canada, India and South Africa. Particular mention might be made of the very active work on fungi in the Union of South Africa, which possesses a good National Herbarium from which a number of excellent monographs have been published. Of the Colonies and Dependencies overseas, Ceylon is the one where fungi have been collected and studied most widely. The early collectors laid the foundations of a good herbarium at Peradeniya, and subsequently the work of a former Government Mycologist very greatly extended our knowledge of the Ceylon fungus flora. Similar work has been begun more recently in other Dependencies, notably Uganda, Sierra Leone, Singapore and Bermuda. Keenly interested Government Mycologists began by sending large collections to this country to be named at Kew, the British Museum and, in later years, at the Imperial Mycological Institute. As a result of this helpful and essential collaboration the overseas mycologists have gained experience which has enabled them to build up their own herbaria and carry out independent monographic work of fundamental importance.

#### THE NEED FOR ENCOURAGING SYSTEMATIC MYCOLOGY

Fungi are among the commonest agents of disease in plants. Thus, in the 1941 *Review of Applied Mycology*, in which the world's literature dealing with diseases of plants (except those caused by animal parasites) is surveyed, over 70 % of the papers on plant diseases abstracted dealt with fungus pathogens.

As has already been pointed out, the study of plant pathology has been greatly developed and encouraged during the past thirty years, and systematic mycology plays a vital part in this subject. It is true that with the development of the Ministry of Agriculture's Advisory Service after the last war, certain posts for 'Advisory Mycologists' were established, but these officers are primarily concerned with plant pathology and not all of them have had the opportunity of making studies in pure mycology. There are now about fifty persons holding posts outside the teaching staffs of universities, who are engaged in work on those aspects of plant pathology concerned with fungi.

Unfortunately, during the same thirty years, there has been little or no official recognition of the importance of the study of fungi as fungi, and the fifty plant pathologists now at work are still largely dependent for advice on and help with their problems in systematic mycology on three already overworked official taxonomists (one at Kew and two at the British Museum), two of whom are approaching the retiring age. Assistance, though necessarily limited, has been given, especially in recent years, by the Imperial Mycological Institute. Nevertheless, as outlined below, the position is very serious and needs immediate attention.

*University training.* The amount of instruction in systematic botany included in the degree courses varies considerably; in English universities it is generally very meagre. Although lectures on fungi and their life cycles are given, there is little instruction in the taxonomy and nomenclature of fungi. The amount of encouragement given to taxonomy depends almost entirely on the interests of the Professor and candidates for higher degrees are rarely, if ever, advised to take up taxonomic problems. One reason for this is undoubtedly that there are so few posts open to taxonomists. It is doubtful if any university provides a course on nomenclature, and the technique of examination of herbarium specimens is generally unknown to students entering applied mycological posts. Courses in general mycology in this country do not compare unfavourably, on the whole, with those, for instance, in America, and a number of Professorships or Lectureships in Botany in our Universities and University Colleges are held by individuals keenly interested in mycology, but these courses do not include adequate training in taxonomy. In our view systematics is an essential part of any problem in phytopathological, industrial or medical mycology. One need is for applied mycologists with sufficient knowledge of the fungi and of the literature concerning them to be capable of identifying the common species which are met with as pathogens, or in industry, or as sources of human and animal food. Such workers would relieve mycologists at the central Herbaria of much routine work, enabling them to turn their attention to much needed revision and research work. If the posts are provided, a demand will be created for post-graduate systematic training in the Universities. This may have to be met in the first instance through the help of the few systematists now holding official appointments. Facilities for post-graduate training in systematic mycology are essential if the present state of affairs is to be improved. It is a sad commentary on the training of plant pathologists in systematics that none of the agricultural or horticultural research stations in this country maintains a working mycological herbarium.

*Central herbaria.* It cannot be too strongly emphasized that research work on a parasitic organism has little permanent value until the organism has been correctly identified and its relationships understood. Failing this, unnecessary confusion and duplication of work may result and the accumulated knowledge of the pathogen under various names will not be fully available. Lack of good advice on systematic mycology, or inadequate knowledge, has undoubtedly led to the literature becoming encumbered with a vast number of names because authors have not been familiar with or have not appreciated previous work. The applied biological sciences,

such as agriculture, horticulture, forestry, medicine, and many industrial undertakings which either use or are affected by fungi, are dependent on qualified taxonomists to whom mycological problems can be referred, as the requisite specimens and literature necessary are centred in the larger herbaria. Moreover, the day is long past when the whole field of the fungi can be covered by one individual. While plant pathology has been developing on the one hand, systematic mycology has also been increasing in extent and complexity. Not only have numerous new genera and species of fungi been recognized in the past few decades, but the increased use of the microscope and of cultural methods by the pathologist have helped to revolutionize taxonomic work. New ideas of the value of both morphological and biological characters are coming into being, which may have far-reaching effects on classification and nomenclature. Careful revisions of certain genera and families of fungi in the light of modern knowledge are urgently required and as fresh knowledge accumulates every group needs repeated revision. To meet all these demands adequate central facilities are necessary, and these can be provided only at the great National Herbaria at the Royal Botanic Gardens, Kew, and the British Museum (Natural History), at the Imperial Mycological Institute and at certain of our Universities. At all these places it should be possible for fundamental research on taxonomic problems to be carried out unfettered by demands for results of immediate economic importance. Such work requires long training, which cannot be included in any degree course. It is no exaggeration to say that ten years' work in a herbarium is necessary before a mycologist begins to acquire the general knowledge and sound judgement which is essential to a good taxonomist.

As already explained, Kew has one mycologist, and the British Museum two, one of whom is also Keeper of Botany. These officers are responsible for the upkeep of collections, for routine correspondence and extensive advisory work, and for assisting workers at home and abroad by the investigations of type specimens, etc. in the collections under their care. Consequently there is little or no time for research, and in this respect England is falling sadly behind in mycological work as compared with North America. The Imperial Mycological Institute has two mycologists working on taxonomic problems, but by the terms of reference of the Institute their work must usually have some bearing on plant pathology, and especially the problems of the Empire overseas. The number of experienced workers is thus dangerously small. Apart altogether from the question of carrying out original work in mycology, it is highly probable that unless steps are taken in the near future to rectify matters, not only will it become increasingly difficult to train students, but it may soon become impossible for the applied mycologist to obtain an authoritative identification in this country of organisms belonging to certain groups of fungi.

*Research Stations and advisory service.* The liaison between the Research Stations and systematic mycology is twofold. When a fungus pathogen, proved or suspected, has been isolated by a pathologist and he is uncertain of its identity, the present procedure is to submit specimens or cultures to taxonomists at the central Herbaria. The systematic specialists at these



institutions are better equipped both by experience and reference facilities for much of this work, and but for their co-operation much valuable time and accuracy would inevitably be sacrificed by the pathologist. The examination of such material, however, places an additional routine burden on the present very restricted number of pure systematists, and the rapid expansion of activities in pathology at the Research Stations clearly calls for some expansion in the staffs at the central Herbaria. Nevertheless, at least some of this work of identification could and should be done at the Research Station itself.

Concurrently the pathologist carries out experiments on the biology and pathogenicity of his fungus, during the course of which a body of valuable and essential data on the effect of host and other environmental conditions is obtained, and this is of the utmost reciprocal value to the systematist for determining new standards in classification and nomenclature. To carry these studies to their final stages an appropriate taxonomist from the central Herbaria might profitably be temporarily attached to the Research Station Pathology staff, in order to make the necessary 'field contact' with the fungi in question. Present experience at Research Stations shows that the pathologist needs a sound grounding in fungus systematics for the efficient elucidation and application of these biological data. Pathologists lacking this background inevitably tend to regard the morphological identity of the pathogenic fungi as of secondary or even negligible importance. To counter this it is felt that an adequate course in systematic mycology should form part of the training of every Research Station pathologist working on fungus diseases, and that full facilities for specializing in taxonomy should be given to those who show leanings in this direction. Such specialization would normally include some months' training at one of the central Herbaria.

The same considerations apply in the liaison between systematic mycology and the provincial Advisory Mycologists. Close collaboration between the central Herbaria and Advisory Mycologists is very necessary, but in addition the Adviser must look to his headquarters at Harpenden for assistance with many of his mycological problems, and to meet this need mycology (as distinct from plant pathology) needs strengthening at the Ministry of Agriculture's Plant Pathology Laboratory at Harpenden.

*Industrial mycology.* All industrial products and raw materials of an organic nature are liable to attack by fungi and almost all manufacturers and merchants of such products experience trouble from mildew or mould at some time. In comparatively recent years much has been done in some industries to develop methods of control, particularly in the cotton industry, in leather and rubber manufacture and in the food canning industries. A very serious hindrance to rapid progress in industrial mycology, however, is the difficulty of identification of the organisms responsible for damage to products. Brewers, distillers, cider manufacturers and wine producers are dependent upon definite strains of yeast and not only must these be maintained free from contamination by wild yeasts of rather close affinity, but also from non-sporulating forms which cause disease. In addition there is increasing use of moulds in fermentation industries. Citric acid is now produced almost exclusively by mould fermentation, and gluconic acid,



lactic acid and tannic acid can be manufactured in similar ways. Fats, glycerol, enzymes, alcohol, vitamins and other desirable products can all be obtained on a large scale by fermentation processes. A recent application is the manufacture of 'food yeast', rich in protein and the vitamin B complex, by growing a non-sporing yeast on molasses. Anti-bacterial and anti-fungal substances, such as penicillin, patulin and gliotoxin, are the products of definite species of moulds. In all these useful applications of microfungi it is essential that the organisms be correctly identified. At present identifications are often carried out by one worker for another as a matter of courtesy. This usually means that an expert in a particular group of fungi, already fully occupied with his own investigations, is overwhelmed with requests for assistance. It also means that the inexperienced worker, who particularly needs assistance, may, from lack of the necessary personal contacts, be unable to obtain the help that is needed. The difficulties could be largely overcome by extending, on the industrial side, the scope of the National Collection of Type Cultures, and it is recommended that a specialist in industrial fungi be appointed to its staff.

*Forest and timber pathology.* Systematic study is essential for the solution of many outstanding problems in forest pathology. Correct naming of organisms responsible for leaf and seedling diseases is difficult, and there is a great deal of confusion even about the larger fungi that cause Heart Rot. Identical fungi in America and Europe have been given different specific names. In timber pathology a similar state of affairs exists and reference often has to be made to American and Continental authorities. In practice a wrong identification may lead to serious consequences, because intensive work carried out on one organism may lead to the adoption of control measures which if applied against a different pathogen may prove useless.

A collection of cultures can be used as standards only if the original isolations have been made from correctly named sporophores. Otherwise great confusion may result. The preparation and maintenance of such a collection should always be in the hands of specialists in the different groups.

*Medical mycology.* The whole subject of medical mycology in this country calls for consideration. There appears to be no course of lectures at any Medical School except a short one for overseas medical men at the London School of Hygiene and Tropical Medicine. So far as taxonomy is concerned the accounts of fungi published in British medical works give clear evidence that some fundamental training is necessary. Compared with what has been done in France, and what is now being achieved in the United States, the amount of sound work accomplished in this country is insignificant. It is understood that the Medical Research Council has recently appointed a Committee to report on the matter and that taxonomy and nomenclature are being considered as a special aspect of the problem. Comment is therefore restricted to the observation that the existence of the National Collection of Type Cultures is of the utmost importance and to emphasizing the need for a medical mycologist on the staff. In obtaining cultures from various investigators it acts as a check on haphazard naming and can provide material for investigation and for comparative studies.

## RECOMMENDATIONS

It is apparent that the difficulties facing systematic mycology in this country cannot be overcome rapidly. There is a great demand for mycologists qualified to undertake taxonomic work. Fungi are not only agents of disease or spoilage: they are also used in many industrial processes, in medicine, and to some extent for food. There is need for mycologists who can identify correctly the species and strains of fungi concerned, who can collect the morphological and biological data essential to the systematist for his taxonomic studies, and who can carry out monographic studies of fungus genera and families in the light of modern knowledge. To meet this need it is recommended that:

(1) The staffs for systematic work at the two National Herbaria, the Royal Botanic Gardens, Kew, and the British Museum (Natural History), and at the Imperial Mycological Institute should be increased sufficiently to ensure a continuity of expert advice and to allow adequate opportunities for research.

(2) Facilities should be provided for the temporary attachment of taxonomists from the central Herbaria to the staffs of institutions dealing with applied mycology when field contact with a particular group of fungi is essential for further taxonomic study. Opportunities should also be afforded for research and advisory pathologists to be seconded to work in the central Herbaria for short periods.

(3) An effort should be made to ensure that plant pathologists are given more intensive training in mycological taxonomy and that they are afforded facilities for specialization in this subject.

(4) At the Plant Pathology Laboratory, Harpenden, mycology should be given increased attention. For this purpose the appointment of a taxonomic mycologist is suggested.

(5) A specialist in industrial fungi should be appointed to the staff of the National Collection of Type Cultures. This would enable arrangements to be made for private firms and research associations to obtain identifications of moulds. Later, the service might be extended to giving advice on industrial problems.

(6) It is suggested also that a medical mycologist should be added to the staff of the National Collection of Type Cultures.

## CORDYCEPS APHODII, A NEW SPECIES, ON PASTURE COCKCHAFFER GRUBS

By J. MATHIESON, *Botany School, University of Melbourne*

(With Plates XII-XIV and 10 Text-figures)

### INTRODUCTION

Cockchafer grubs have always caused a certain amount of injury to pastures in Victoria during the winter, but in recent years the damage done by the common Pasture Cockchafer, *Aphodius howitti* Hope. has become more severe, and in the winter of 1942, pastures were very seriously damaged in certain parts of Victoria. (Twentyman & Pescott, 1942). In general, subterranean clover pastures have been most damaged, the grubs destroying the whole plant, and the pasture coming to look as though it had been ploughed.

During the last two years, officers of the Department of Agriculture, who have studied the grubs in the field, have noticed that dead larvae in the soil often showed evidence of fungal attack. Before it is possible to assess the value of the fungus as a means of biological control, it is necessary to know its life history and the conditions which favour its development and spread in the field.

This paper deals only with the life history of the fungus, and gives some account of the biology of the parasite in the host.

### LIFE HISTORY OF THE BEETLE

The beetles are dark brown, about half an inch long, with longitudinal parallel striations on their wing cases. They emerge in late January or early February and they gather together in myriads round exposed lights, and may also be found in enormous numbers in patches of dung in the fields. The cream-coloured eggs are about  $\frac{1}{8}$  in. long and are laid in groups of thirty to forty in the ground in small earthen cells. After the larva emerges the sticky egg case adheres to the body as a shrivelled skin, but is soon lost as the grub moves through the soil. The length of the incubation period is not known accurately, but seems to be about three weeks.

The young larvae are only  $\frac{1}{8}$  in. to  $\frac{1}{4}$  in. of an inch long when they hatch, and the body remains white until they have their first meal of earth. The head is at first pale brown, but changes to dark brown within six to twelve hours. The rate at which they grow depends on the season; full-grown larvae have been found in April, but during the drought years 1943-5, when the rainfall was 24.05, 22.75 and 22.03 in. respectively (average annual rainfall 27.22 in.), the grubs were not full grown until well into May. When fully grown, the larvae are approximately 22 mm. long and 4-5 mm. broad. They are greyish white owing to the dark contents of the gut being visible through the transparent skin, and the head is dark brown to black;

there is also a distinguishing pattern of hairs on the last segment of the abdomen.

At first, if the soil is dry, they feed only on roots, but, after the first autumn rains, the ground becomes soft enough for them to tunnel through it and they begin to feed on the foliage. They emerge at night and frequently carry a supply of leaves down into their tunnels where they remain during the daytime.

Most of the grubs continue feeding through the winter until about the end of August, when they pass into the non-feeding pre-pupal stage and grubs in this state are readily distinguished by their milky-white bodies. They remain in the soil in earthen cells until they pupate in October, finally emerging as the adult beetle at the end of January.

#### THE *CORDYCEPS*

Three different types of fungal growth were found on the dead larvae; the stromata of the *Cordyceps* and two stilboid forms, one of which was white and slimy, and the other at first white but rapidly developing areas of a waxy consistency which changed from bright yellow to an olive green. Although there was a strong possibility that these stilboid forms represented the conidial stage of the *Cordyceps*, they have been found to be parasitic on its stroma. The former is *Tilachlidium brachiatum* (Batsch.) Petch, and the latter belongs to the family Discellaceae of the Sphaeropsidales.

#### (1) *Morphology of the Cordyceps on the host*

##### (a) *Materials and methods*

Supplies of infected and uninfected larvae have been provided by Mr Twentyman of the Department of Agriculture, chiefly from Miner's Rest, near Ballarat, Victoria.

Stages in the development of the endosclerotium were studied from paraffin sections. The larvae were fixed in C.R.A.F. and in Carnoy's fixative, dehydrated, cleared in chloroform and embedded in paraffin. Sections were cut 7-10  $\mu$  thick and stained with Haidenhein's iron-alum haematoxylin and counter-stained with erythrosin.

For the development and structure of the asexual and sexual stages of the fungus, both hand sections and paraffin sections were used. Temporary mounts were made in lacto-phenol and stained with cotton blue. Paraffin sections were prepared as described above, after the material was fixed in Fleming's weak fixative.

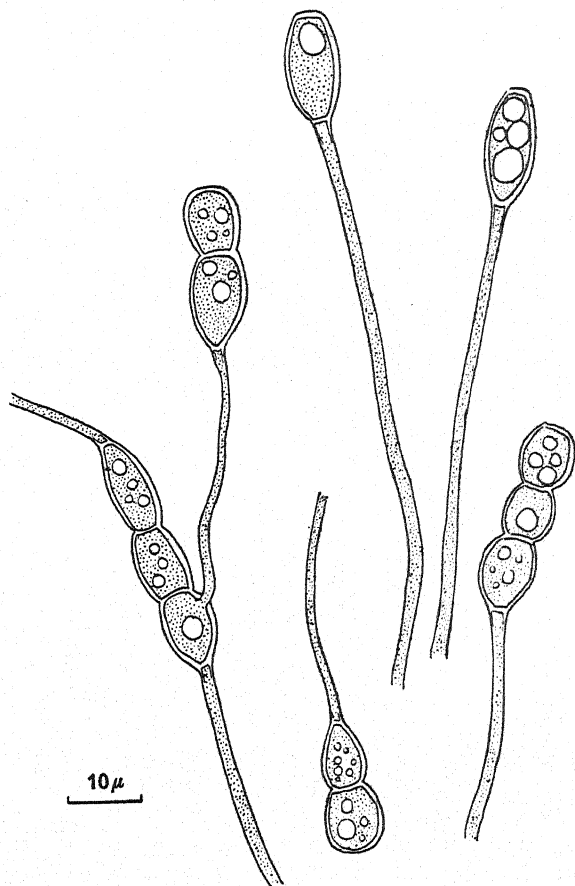
Dead and infected larvae were planted on the surface of non-sterile soil in large 5 in. Petri dishes, about ten larvae per dish, and incubated at room temperature and at 24° C.

The following account has been compiled from observations on larvae in the field, from naturally infected larvae which have been incubated in the laboratory, and from sections of infected grubs and stromata.

##### (b) *The Endosclerotium*

The external form of the larva remains unaltered after the formation of the endosclerotium, but the colour changes to either pinkish white or buff,

the skin toughens and the body shrinks and becomes opaque. The interior is completely packed with a whitish mycelium, and the mass eventually becomes firm and often quite hard. The mycelium consists of extremely fine hyphae and gemmae. The gemmae are occasionally globose but usually oval with flattened ends; they may occur singly or in groups of two or three together, and may be terminal, intercalary or lateral to the hyphae. They are  $7.5-15\ \mu$  long and  $6-8\ \mu$  wide, and the hyphae are about  $2\ \mu$  wide.



Text-fig. 1. Hyphae and gemmae from the endosclerotium.

(c) *The stromata*

As soon as the formation of the endosclerotium is complete and if environmental conditions are favourable, clubs begin to develop from the exterior of the body of the larva (Pl. XII, figs. 1, 2). These appear first as small cushions of mycelium on either side of the head and very quickly elongate to a length of about 1-1.4 cm. The length of the club varies according to the position of the grub in the ground, but in the field, the

variation is very small, as most dead infected larvae are found in the upper half inch of soil.

Nevertheless, under certain conditions, clubs will grow longer than 1.4 cm. Infected larvae which were buried beneath one and a half inches of soil in the laboratory developed stromata up to 5 cm. in length, but they were very contorted and grew horizontally, so that none of them reached the surface. During growth they are positively phototropic, and it is conceivable that light controls the growth in length.

The number of stromata produced by a sclerotium varies from two to eleven with an average of five. Two of them usually grow from each side of the head, but others may arise from any part of the body. They are terete, about 2 mm. in diameter and taper towards the tip; usually they are unbranched, but occasionally short laterals may develop.

The stromata vary in colour according to whether they are completely sterile or bear one or other of the two conidial stages. It is unusual to find a sterile stroma developed from sclerotia in the laboratory, but often in specimens collected in the field the conidiophores of conidial stage (A) have been either knocked off or removed by predators and the stromata are naked. Then they are warm brown (between Buckthorn Brown, Dresden Brown, Cinnamon Brown and Russet\*), with pale pinkish brown tips (between Avellaneous and Wood Brown).

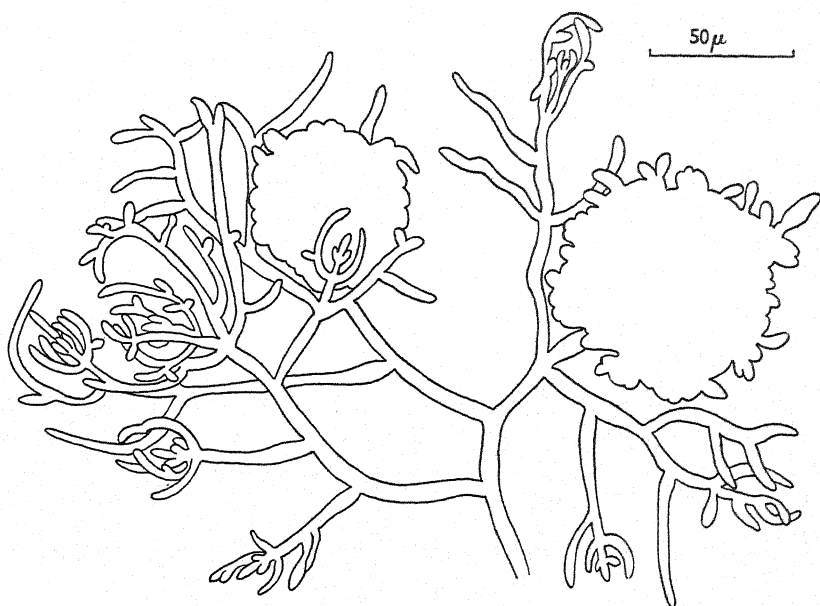
Sections of the stromata show that they consist of a central core of brown longitudinally arranged hyphae and a very narrow outer layer where the hyphae are darker brown and arranged more or less at right angles to the inner. Stromata developed from infected larvae in the laboratory never remain sterile, one or other of the conidial stages always developing, the commonest being conidial stage (A).

(d) *Conidial stage (A)*

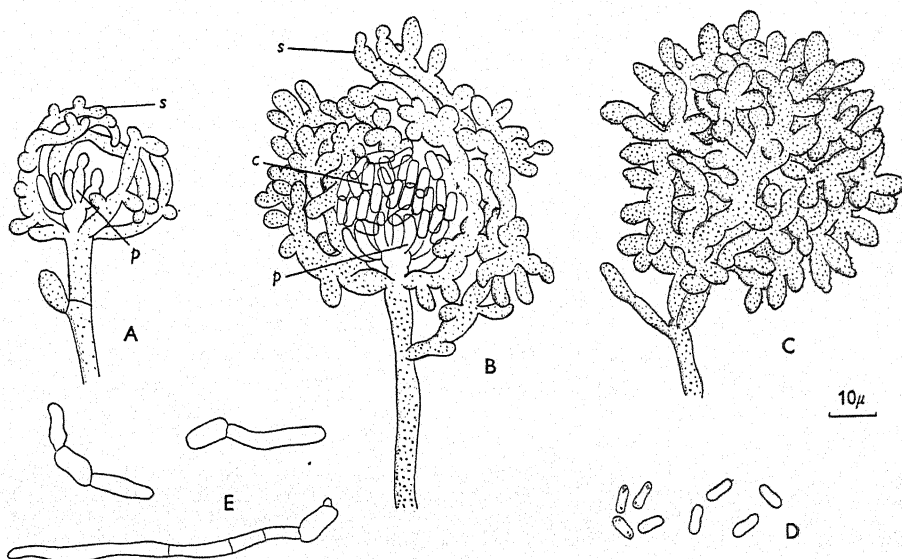
This stage may begin to develop from stromata when they are very small, often little more than cushions of mycelium showing on the outside of the sclerotia. It is not restricted to the stromata, and may develop from anywhere on the surface of the endosclerotium, even from the tips of the legs and along the hairs.

At first, coarse colourless hyphae grow out from the surface of the stroma. Each hypha branches monopodially and conidiophores develop at the ends of the branches (Text-fig. 2). At the extreme tip of each branch, several very short blunt phialides arise, which are hyaline, non-septate and about  $6.25\mu$  long. At the same time, immediately behind these, and for a short distance back along the branch, a number of laterals develop and, becoming sickle-shaped, curve round the apex, at the same time producing many short blunt branches on their convex side. Colourless spores are budded off from the phialides until they completely fill the cavity enclosed by the outer sterile hyphae. The spores are oval, smooth-walled, unicellular bodies, and measure  $5.0-7.5\mu$  long, and  $2.0-2.5\mu$  broad. The sterile hyphae are  $3.75\mu$  wide, and markedly tuberculate. At first they are colourless, but at maturity become buff-coloured, so that the whole stroma, when covered

\* The colours quoted are from Ridgway's Colour Standards.



Text-fig. 2. Conidiophores of conidial stage A, of *C. aphodii*. The mature conidiophores are only shown in outline.



Text-fig. 3. Structure of conidiophores of conidial stage A of *C. aphodii*, and conidia. A, young conidiophore; B, mature conidiophore in optical section; C, external appearance of mature conidiophore; D, conidia; E, germinating conidia. c, conidia; p, phialides; s, sterile hyphae.



with these conidiophores, is Ochraceous Buff to Ochraceous Tawny (Text-fig. 3; Pl. XII, fig. 4).

When the conidiophores are mature, i.e. the spherical heads are full of spores and have changed colour, the stalks wither and the conidiophores remain adhering to each other by virtue of the roughness of the sterile hyphae. If conditions are favourable, this layer may be pushed off by the growth of another crop of conidiophores beneath the old ones (Pl. XII, fig. 5). It is doubtful if they persist thus under natural conditions, as they are probably carried away by small animals crawling over the clubs. Although stromata from larvae collected in the field are sparsely covered with conidiophores, a new crop rapidly develops after incubation in the laboratory for two to three days.

(e) *Conidial stage (B)*

A second conidial stage may develop on the same stromata. Instead of being covered with the whitish, pubescent growth of stage (A), the stromata remain quite smooth and change in colour from Ochraceous Tawny to between Raw Sienna and Antique Brown. If the surface of the stroma is examined with a lens it is found to be finely velvety. This is due to the presence of a palisade-like layer of phialides arising from the central longitudinally running hyphae of the stroma. In section, the colour of the central region is close to Raw Sienna, and the layer of phialides is almost Russet. The phialides are simple, smooth and are slightly broader towards the apex; each one terminates in a fine sterigma. They are  $13.0\ \mu$  long and  $2-3\ \mu$  broad at their greatest width. Each sterigma bears a very slender, pip-shaped spore. The spores are hyaline, smooth and measure  $5.0\ \mu$  long and  $1.25\ \mu$  wide (Text-fig. 4).

When examined in water under the microscope these spores appear to have a thick hyaline wall, although the outer boundary is very difficult to see. When stained with cotton blue the protoplasm of the spore stains, but the wall remains colourless and badly defined. The spores were also stained for twenty-four hours with 0.1 % aqueous solution of Ruthenium red, but the wall still remained colourless. It is difficult to determine whether the spores are dry or coated with mucus. If spores were produced successively and the walls were coated with mucus, the spores would collect in a head as in a *Cephalosporium*. It is not known whether a sterigma produces more than one spore, but there is never more than one present at any given time.

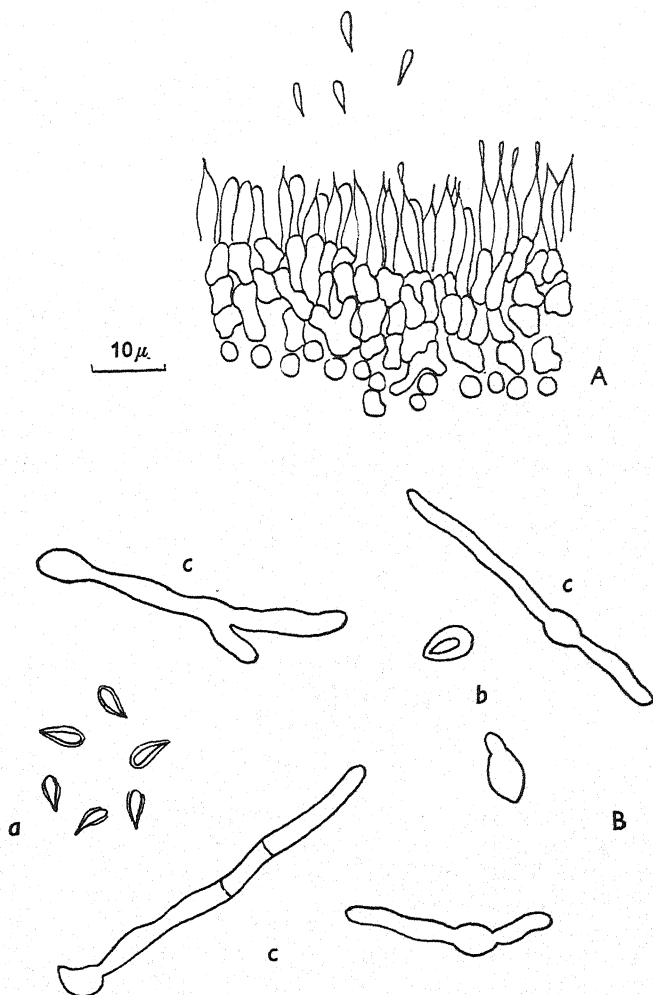
If the spores are dry then this stage belongs to a genus of imperfect fungi—*Hymenostilbe*, described by Petch (1931). A number of species of this genus has been described as conidial stages of species of *Cordyceps*.

Further observations on the fungus in the field are necessary before it can be decided what part this stage plays in the life history, but only once has it been found on stromata which have developed in the field, although it occurs frequently on stromata grown in the laboratory.

(f) *The perfect stage*

This develops on the same club as the two conidial stages. The fertile part is at first intercalary, but may become terminal if the pointed sterile apex develops a few perithecia. It is ovoid to cylindrical and is usually

3-4 mm. long and about 1.5 mm. broad. The colour is the same as that of the rest of the club, and when this stage developed on incubated sclerotia in the laboratory, conidial stage (B) was often present between the ostioles.



Text-fig. 4. Conidial stage B of *C. aphodii*. A, T.S. of stroma showing phialides and conidia; B, germinating conidia. a, conidia before germination; b, conidia swollen prior to germination; c, germinating conidia.

The surface of the fertile part is punctate and slightly papillate, the ostioles, which are dark reddish brown, projecting a short distance above the surface of the club (Pl. XII, figs. 6, 7).

Sections of the fertile head show that it consists of three well-marked layers: (1) a central columella where the hyphae are arranged longitudinally, (2) a wide layer surrounding the columella which forms a spongy

matrix in which the perithecia are embedded, and (3) a narrow outer layer of short hyphae arranged at right angles to the main axis. The perithecia are arranged obliquely to the columella and most of them have upwardly curved necks (Pl. XIII, figs. 1-4). They are flask-shaped and have a dark brown wall consisting of several rows of cells. The average measurements of the perithecia are  $360\mu$  long and  $110\mu$  broad. The wall is  $6.5\mu$  thick.

In this genus there are no paraphyses and the asci completely fill the perithecium. The asci are  $200-225\mu$  long and  $3.8-5.0\mu$  broad. Each has a thick hyaline cap about  $8.0\mu$  broad and  $5.0\mu$  long, crossed by a very narrow canal (Text-fig. 5). The asci do not all mature at once, and spore discharge may continue for several days.

The ascus contains eight long thread-like ascospores which are slightly coiled round each other. As they mature, septa become visible and just before the ascospores are shed, constrictions develop at each septum. The ascospores vary in length from 90 to  $151\mu$ , average  $116\mu$ . They are shed through the ostiole of the perithecium and, if the atmosphere is dry, are carried away by air currents. If the atmosphere is moist, they collect round the mouth of the ostiole like cotton-wool. As they are shed they may break up into lengths if not into the individual part spores, but if water is present they separate readily. The part spores are oval but flattened slightly at each end and are  $5.5-7.5\mu$  long and  $1.25-2.0\mu$  broad.

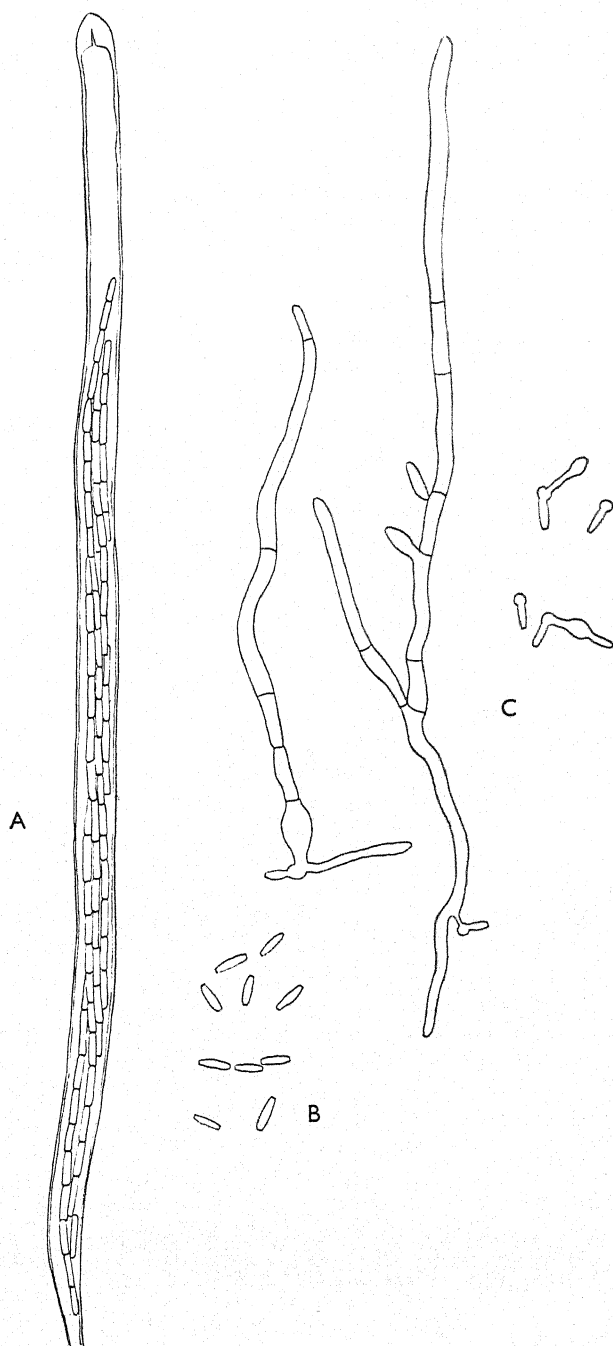
Following the scheme of classification proposed by Kobayasi (1941) for species of this genus, the characters of this species place it at once into the subgenus *Eucordyceps*, although the obliquely placed perithecia are rare in members of this group. Because of this character it comes into the section *Cremastocarpon*, and the subsection *Carnosae*. It differs from any of the species already described in this section in the species of host, the dimensions of the perfect stage and in the nature of the associated conidial stages. Therefore it has been named ***Cordyceps aphodii* n.sp.**

#### (2) *The disease in the field*

Infected are distinguished from healthy larvae by the colour of the body, which changes from the usual greyish white of an actively feeding grub, or milk-white if in the pre-pupal stage, to a faint buffy yellow often with a pinkish tinge, and the skin becomes opaque and more wrinkled. At first these symptoms may be localized in the fore-part of the body, but they gradually spread and intensify until death occurs. The incubation period is not known with certainty, but lies somewhere between a fortnight and two months.

Infection may take place any time from hatching in February until August, when the grubs have all passed into the pre-pupal stage. After this, no grubs in the early stages of infection were ever found, which suggests that either the grubs in this condition are resistant to infection, or since they are inactive, are not exposed to it.

From April to August conidial stage A is always found on stromata in the field and develops abundantly on grubs incubated in the laboratory, but conidial stage B, though developing freely in the laboratory, was only found once in the field. The perfect stage began to develop on incubated



Text-fig. 5. A, ascus containing mature ascospores; B, part spores; C, germinating part spores.

grubs at the beginning of August and by October development was complete; at the same time, mature perithecia were found in the field.

The significance of the ascospores in the life history of the parasite is obscure, unless they remain viable for some months in the soil, because at the time they are shed the grubs are all in the pre-pupal stage and unlikely to be exposed to infection. It is possible, though not very likely, that the ascospores germinate and grow saprophytically in the soil until the young grubs hatch out in the autumn.

During the summer months the clubs shrivel, but they appear to be able to revive again under moist conditions. One specimen, which had been dried for twelve months, was planted again on moist soil; the clubs revived and added 4 mm. to their length, as well as developing conidial stage A.

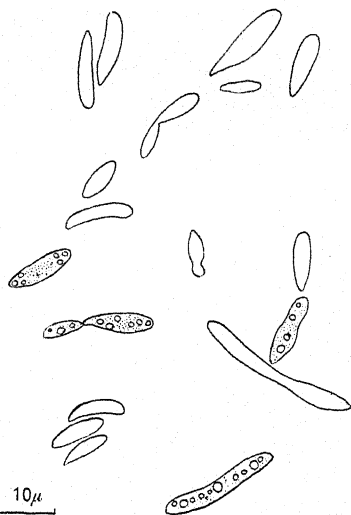
In February 1947, after one and half inches of rain, old stromata were found which had revived and produced a new crop of spores of stage A. Such stromata may provide the source of infection for the new season's larvae.

### (3) *The development of the parasite in the host*

The method of entry of the parasite into the host is unknown, but examination of sections of infected larvae suggests it does not gain entrance through the alimentary canal as it is never present in any part of the gut, nor in the cells of its lining. The other means of entry are through the skin, or the tracheal tubes. Since it has not been possible to infect larvae with the fungus artificially, it has been necessary to select individuals at random for sectioning, as in the initial stages of infection diseased are indistinguishable from healthy larvae.

The fungus is present in the host in the form of hyphal bodies (Text-fig. 6). These are oval-fusiform yeast-like cells, which are usually unicellular but occasionally once-septate, and they reproduce by budding. A number of apparently healthy larvae were sectioned and in several of them these hyphal bodies were present, but in none of them was there any sign of mycelium or any indication of how the parasite entered the body of the host.

The larva of *Aphodius* has a large and much-coiled gut which is surrounded by a haemocoel in which the rest of the tissues and organs of the body are situated. The head is occupied very largely with striated muscle which is also well developed throughout the rest of the body. Early in life the fat body is small, but it gradually increases in size until at the pre-pupal stage it occupies almost the whole of the body cavity. The spiracles are situated



Text-fig. 6. Hyphal bodies from an infected larva.

along both sides of the body, and each leads into a cavity from which radiate the tracheal tubes.

In the earliest stages of infection which have been examined, unicellular hyphal bodies were present throughout the haemocoel, and on the walls of all the organs in contact with it. They were clustered in large numbers round the tracheal tubes especially (Pl. XIV, fig. 2), and round the muscles in the head. They were also present on the inside of the epidermis, round the fat body, the body muscles, the Malpighian tubes and on the outer surface of the wall of the gut. Such is the distribution at the first outward signs of infection, although by this time the numbers of hyphal bodies present have increased enormously. As the symptoms spread to all parts of the body a change takes place in the character of the parasite; instead of the daughter cells separating as before, they remain attached to the parent and small chains of cells develop which come to occupy the whole body cavity. They are clustered in tremendous numbers round the gut which by this time is considerably compressed, and are also present in and between the fat body (Pl. XIV, fig. 3), but the cuticle and muscles are still intact. It is at this stage that death occurs. The body of the host is still quite soft and further development of the parasite takes place before the formation of the mature endosclerotium. The chains of cells give rise to very fine hyphae which penetrate the skin, the muscles and the fat body, so that although these structures are recognizable in the body of the larva, they are completely permeated by the fungus. The hyphal chains therefore become the typical gemmae of the sclerotium.

Hyphal bodies similar to those found in *Aphodius howitti* have been described by de Bary (1884) for *Cordyceps militaris*, and by Petitt (1895), for *C. clavulata* in scale insects. They both described them as yeast-like bodies, and Petitt also describes some in *Lecanium fletcheri* which were larger and once or twice septate, possibly merely the same bodies at a later stage of development. De Bary states that the part spores germinate on the skin of the caterpillar in *Cordyceps militaris*, which they penetrate at any point on the surface and develop a mycelium of stout hyphae which penetrate the muscles and fat body. Mycelial growth then stops and instead short 'cylinder-gonidia' are cut off from the ends and sides of the hyphae and set free into the blood stream. These are the hyphal bodies. No trace has been found in *C. aphodii* of any initial mycelium from which the hyphal bodies develop, nor of the means of entry of the fungus into the host.

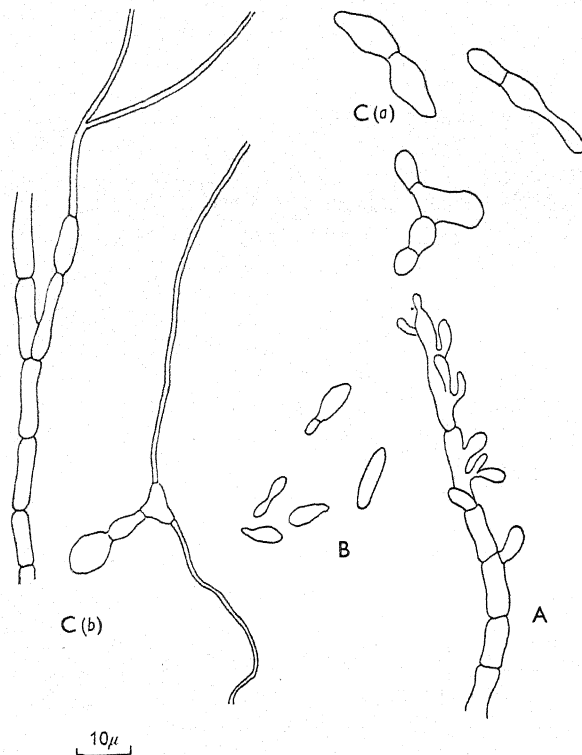
#### (4) *The Cordyceps in artificial culture*

##### (a) *Materials and methods*

Sterile cover-slips were coated with a thin film of sterile, filtered, melted agar. A spore suspension was made in water and a few drops spread over the surface of the agar. The cover-slips were then incubated in sterile Petri dishes lined with moist filter-paper. The cover-slips were examined at intervals aseptically through the back for germinating spores. Single spores were then cut from the agar using a cutter which fits over the low-power objective and has the diameter of the low-power field. The blocks of agar bearing single germinated spores were then transferred to agar plates.

Potato-dextrose agar was found to be the best medium for germination of spores and subsequent growth of the colony.

Cultures were also made on the larvae themselves. They were killed by exposing to chloroform vapour, then placed on a pad of moist cotton-wool in a test-tube and autoclaved for fifteen minutes at one atmosphere. Cereal grains were used in an attempt to induce the formation of the perfect stage. Rice, barley and wheat grains were saturated with water and autoclaved in flasks.



Text-fig. 7. A, hyphal bodies developing on a hypha in liquid medium. B, detached hyphal bodies. C (a), chains of cells; (b), types of mycelium developed in culture.

#### (b) Germination of ascospores

Fertile clubs were suspended over sterile cover-slips inside a sterile Petri dish overnight and a heavy deposit of part spores was obtained. A spore suspension was made in sterile water and sown on thin films of nutrient and potato-dextrose agar. Germination always occurred within three days at 24° C. A swelling was first produced at one end of the spore and later a germ tube developed from this (Text-fig. 5C). Growth ceased at this stage on nutrient agar, but on potato-dextrose normal germ tubes were produced, very much broader than the part spore, and a mycelium was developed. Single germinated part spores were transferred to plates of potato-dextrose agar and incubated at 24° C.



The fungus is particularly slow-growing and the colony produced is very restricted. Visible growth from a single spore appears after seven to ten days as a tiny mound of rather moist-looking mycelium which is faintly tinged with pink. Growth continues at approximately the same rate both vertically and horizontally. Most of the mycelium is submerged and this submerged growth gradually builds up until a convoluted mound of mycelium is produced. A very short, whitish pubescent growth of aerial hyphae develops on the surface of the mound, and surrounding the latter is a narrow ring of submerged mycelium. The colour of the colony is between Ecru Drab and Light Cinnamon Drab, and the reverse varies from between Vinaceous Buff and Avellaneous to Deep Brownish Drab and Dusky Drab. At this stage, i.e. eighteen to twenty-one days, conidial stage A may develop throughout the aerial hyphae although the period which elapses before this stage is produced varies considerably. One factor seems to be the depth of medium (and therefore presumably the maturity of the colony) as it appears more quickly in Petri dish cultures than on slopes.

On slopes after six weeks there may still be no sign of conidial stage A and the colony deepens in colour to Pale Purple Drab while the medium is Light Cinnamon Drab. At this stage, parts of the surface of the colony may change in colour to between Deep Colonial Buff and Chamois. Eventually, spherical conidiophores of stage A are produced usually on localized parts of the surface of the mound and the colour then changes to between Buckthorn Brown and Antique Brown. Stromata develop five weeks after inoculation of a plate, but on a slope the time is correspondingly longer. The number varies from one to ten, and they are either sterile or bear the spherical heads of conidial stage A, but never stage B nor the perfect stage. The colony is now mature, and no further growth takes place even when the entire growth is transferred to fresh medium. The maximum diameter is about 15 mm., and the colour of the reverse has changed to between Saccardo's Umber and Bister.

Microscopic examination of a young colony shows that it consists of a mass of coarse hyphae closely septated, from 3 to 4  $\mu$  wide, and with some very fine branches about 1  $\mu$  wide. In addition, there are short lengths of hyphae consisting of two to four irregularly swollen cells which develop as laterals on the main hyphae and then become detached; these may in turn give rise to long fine hyphae. The whole mass may be up to 5 mm. thick, and is of a leathery consistency. Later, conidial stage (A) develops amongst the aerial hyphae in the same way as on the stromata or the sclerotia.

(c) *Germination of spores of conidial stage A*

The heads were crushed in water so that the spores were liberated. These were then sown on potato-dextrose agar according to the method described above for isolation of single spores. Germination was extremely slow even at 24° C. In one case germination occurred after five days and in others between seven and nine days. The spores swell considerably in size, and germinate by a single germ tube or by one from each end (Text-fig. 3). The hyphae are coarse and closely septate. The colony produced is identical with the colony produced by part spores.

(d) *Germination of spores of conidial stage B*

Germination occurred on potato-dextrose agar after four days at 24° C. The spores swelled enormously, becoming quite oval, and developed either one or two germ tubes, one from each end of the spore (Text-fig. 4). The hyphae are coarse and closely septated, and the colony formed is identical with that from part spores.

(e) *Growth from hyphal bodies*

It was found (see section 3) that the blood of living larvae which were suspected of being infected was swarming with yeast-like hyphal bodies. Cultures were made from these hyphal bodies to compare the type of colony produced with that from part spores. The skin of a living infected larva was pierced with a needle, and the liquid which exuded was streaked with a sterile loop over the surface of a potato-dextrose agar plate and incubated at 24° C. Growth was very slow and was hardly visible after a week. Under the microscope it was obvious that the hyphal bodies had grown, but instead of budding as they do inside the larva they had developed short lengths of swollen bead-like hyphae, some of which had produced fine hyphae-like germ tubes from either end. In others, these fine hyphae had become septate and developed another row of swollen cells on the other end. As growth continued, a colony which was similar in character to that from part spores was formed, finally producing conidia of conidial stage A.

(f) *Growth on other media*

(i) *On sterilized larvae.* Several larvae were inoculated with a suspension of part spores. The skin was lightly pricked with a needle, and a drop of liquid exuded. The part spores were sown in this liquid. After twelve days at 24° C. the bodies of the larvae had become pink and a whitish pubescence was apparent at the point of inoculation. They finally became buff-coloured like the sclerotia collected in the field, and developed stromata and conidial stage A (Pl. XIV, fig. 3).

(ii) *On cereal grains.* Kobayasi (1941) obtained fertile stromata of *C. militaris* on sterilized moistened rice grains. An attempt was made to do the same with *C. aphodii*, using wheat, barley and rice grains. Flasks of each were inoculated with a suspension of ascospores, and after sixteen days at 24° C. the rice grains began to show a pinkish discoloration and a very slight whitish down could be seen round the inoculum. Between four and six months after inoculation the rice grains were completely permeated with mycelium, and small slender stromata had formed, which were covered with conidial stage A, but there was no sign of perithecia. After six months the barley and wheat grains were also permeated with mycelium, but only two small stromata had formed.

(iii) *On liquid potato dextrose.* Since growth of hyphal bodies on a solid medium gave rise to a mycelium and not to more hyphal bodies it was thought that since development takes place in a liquid medium, the blood, this type of growth may be a natural response to the type of medium. Therefore liquid potato dextrose, having the same composition as the

potato-dextrose agar, was inoculated with spores of conidial stage A. The pH of the blood was found to be between 6.0 and 7.0, so the medium was adjusted to both pH 6 and 7, and both sets were sown with spores. The spores germinated, and the colonies were at first submerged, developing as small spherical masses of mycelium. Later some of these reached the surface of the liquid and assumed the usual form of a colony on potato-dextrose agar. They were found to consist of hyphae and hyphal segments like the colony on agar, but large numbers of hyphal bodies similar in shape and size to those found in the host were also present in the medium. They are produced in clusters along the coarse hyphae and being quite narrow at their point of origin are easily detached. They then continue to grow by budding.

#### (5) *Infection experiments*

Having isolated and grown the parasite in pure culture, the next step was to reinfect the host larvae. Unfortunately all efforts to do this have failed, owing mainly to the fact that handling of any sort, however gentle, seems to damage the larvae, and there is a very high mortality. It is possible, also, that the conditions under which the larvae were kept in the laboratory were unfavourable, but the fact remains that from two experiments, using over 1000 living larvae, so few survived that any results were useless. The death of the larvae was obviously not due to the parasite as after death they usually turned black and disintegrated, instead of forming a sclerotium, and death was always too rapid.

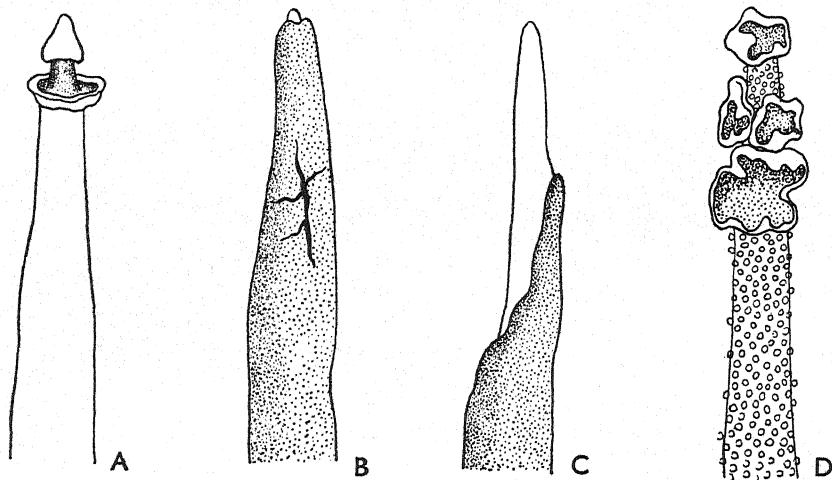
#### OTHER FUNGI FOUND ON THE SCLEROTIA IN THE FIELD

In addition to the *Cordyceps*, two other fungi are associated with the sclerotia in the field. Both of these may occur as stilboid outgrowths from the sclerotium, and at first it was thought that they may be the conidial stage or stages of the *Cordyceps*. It has been shown, however, that they are both parasitic on the sclerotium or on the actual stromata themselves. As well as occurring in the field, sclerotia incubated in the laboratory on moist soil invariably develop one or another, and sometimes both, of these fungi.

#### (1) *A species of the family Discellaceae*

This may first appear as small tufts of white hyphae arising from the sclerotium. These rapidly elongate and form white clavae up to 2 cm. but usually 0.5–1.0 cm. high, which may or may not be branched, and may remain cylindrical or spread out fan-wise (Pl. XIV, fig. 1). Sooner or later during their development a yellow waxy layer develops in various positions on the club. It may completely envelop the white clava leaving a tiny white tip projecting at the apex, or it may develop in localized areas. Often it encircles the clava near the apex and at its lower boundary the white hyphae of the clava grow outwards forming an upturned flange with the yellow layer lining its inner and uppermost surface; then at its upper edge another smaller downwardly directed flange develops and the clava ends in a small white pointed tip. The whole structure resembles a minaret.

Many variations of this form may occur. Sometimes the clava is short and stumpy and the waxy layer covers the whole structure except for a white cup at the base and a white cap at the apex. As well as developing on its own clavae, it may form on the stromata of the *Cordyceps*. Here it takes the form of cup-shaped sporodochia with crenulate edges, which develop laterally on the clavae. The outer surface of the cup is white and the inner is lined with the yellow waxy layer (Text-fig. 8, Pl. XIV, fig. 2). As the clavae age, the yellow layer may alter in colour to a deep olive green.



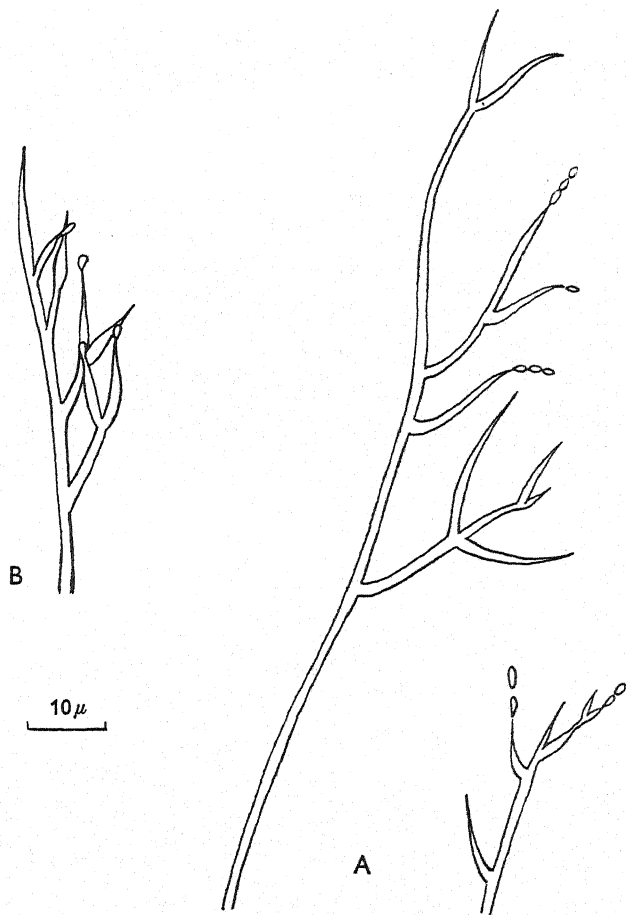
Text-fig. 8. Types of fruiting bodies of parasite (1).  $\times 4$ . A-C, the dotted part represents the yellow waxy areas; D, open 'pycnidia' of this form on a stroma of *C. aphodii* covered with conidial stage A.

The clava consists of a central region of longitudinally running hyphae, and in the white part of the clava the outer hyphae curve outwards and develop conidiophores at their apices. These arise from a main hypha and may be unbranched, dichotomously or trichotomously branched (Text-fig. 9A). They are slender and about the same width as the hyphae, but taper towards the tips. Conidia are produced in chains at the tips of the branches and are smooth, hyaline, oval in shape, and measure  $1.25\mu$  long, and  $2.5-3.0\mu$  broad.

In the yellow waxy areas the conidiophores form a very compact palisade layer at right angles to the central core. The conidiophores resemble those in the white areas but are not so lax and the phialides are more regular in their formation (Text-fig. 9B). The lateral branches all develop on one side of the main hypha, and are usually bifurcate but may be unbranched. The spores are smooth, hyaline and globose, measuring  $1.25-1.9\mu$  in diameter. They are produced in enormous numbers and accumulate in a thick layer above the conidiophores. At first the whole mass is enveloped in mucus, but this gradually dries out and the spore layer becomes waxy.

Since the spores produced by these areas differed in shape and in the method of production, i.e. one in chains and the other in masses, it seemed

possible that they may belong to different fungi, and that one was parasitizing the other. Spores from each area were sown in the manner described above for single spore isolation. After two days, spores from the white area had germinated and produced long germ tubes. Those from the



Text-fig. 9. Conidiophores of parasite (1) (Discellaceae). A, conidiophores from white clava; B, conidiophores from the yellow waxy area.

yellow area had just begun to germinate. A number of single germinated spores were isolated and plated out on potato-dextrose agar. Exactly the same type of colony was produced by each type of spore.

At first a very short white aerial mycelium develops on the surface as the submerged hyphae spread out from the inoculum. This consists almost entirely of conidiophores and conidia of the 'white' variety, but in some of the conidiophores the spore chains have collapsed and the spores adhere in a spherical mass to the tips of the phialides like a *Cephalosporium*. Gradually

small circular areas develop which are cream-coloured and covered with liquid. These may coalesce, forming a more or less continuous cover, and as they mature, darken in colour to bright maize yellow and finally become waxy. These areas consist of conidiophores of the type found in the yellow waxy areas under natural conditions. At about one centimetre from the centre of the colony a ring of clavæ develop which at first are white but eventually are covered entirely or in part with the yellow waxy coat so typical of this fungus. Growth then continues horizontally as before with mixed white and yellow areas, then another centimetre further away from the inoculum another ring of clavæ develops (Pl. XIV, fig. 3). On potato-dextrose agar the diameter of the mature colony is 2.6–2.7 cm. The reverse is brown.

The order of the Fungi Imperfecti into which this form is placed would vary according to which particular form of sporing body one regards as typical. If the open cup-shaped structures, lined with a thick moist mass of conidia, which develop on the stromata of *Cordyceps* and on the endosclerotia, were the only fruiting bodies developed, the fungus would be classified in the Discellaceae, a section of the subfamily Patellinae.

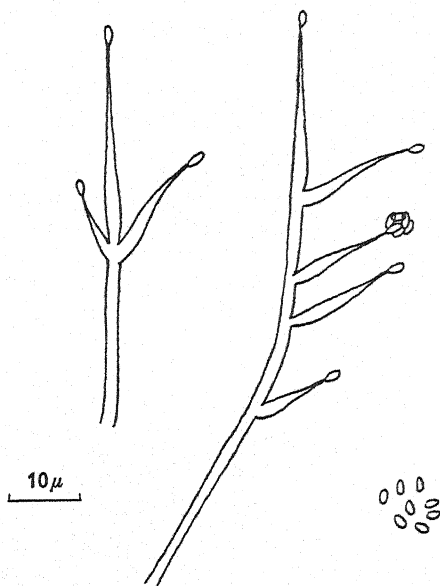
Sometimes only the white and yellow synnemata are developed and then it would be better described as a stipitate *Tubercularia*. In fact, Petch (1939) describes a fungus on sclerotia of a *Cordyceps* on 'white grubs' which has similar white and yellow clavæ although with conidiophores of *Gliosporium* and calls it a stipitate *Tubercularia*. The most constant features are the type of conidiophore and the slimy nature of the spores, and it would seem more reasonable to classify the fungus on such characters rather than on the form of the fruiting structure when the latter is so variable. But according to the present system of classification the most convenient systematic position, i.e. one which would facilitate subsequent identification, is in the Discellaceae, subfamily Patellinae, as this is the form in which it is found most frequently under natural conditions.

## (2) *Tilachlidium brachiatum* (Batsch.) Petch

The second fungus also develops from the sclerotium as white tufts of hyphae which eventually elongate forming groups of white clavæ, up to 7 mm. in height. Each clava bears numerous fine synnematal branches which give a coralloid appearance to the whole growth. The clava and its branches are covered with *Cephalosporium* conidiophores; each head is enveloped in a drop of mucus, and as the heads develop these drops frequently coalesce and the whole clava is covered with mucus and becomes cream-coloured (Pl. XIV, fig. 4). The phialides are simple and arise as laterals from a main hypha, sometimes two from one level. They are slender, slightly inflated towards the base and tapered to a fine tip. The spores are smooth, hyaline and oval,  $0.75\text{--}3.0\ \mu$  long, and  $0.5\text{--}1.75\ \mu$  wide (Text-fig. 10).

Single spore cultures were grown on potato-dextrose agar. After twelve days the colony measures about 3 cm. in diameter. At first it is white but later ranges from Light Buff to Pale Pinkish Buff. The surface is covered with hundreds of tiny synnemata which vary in height from 0.4 to 1.0 mm., and some of them bear very fine branches. In addition to these small

synnemata, which develop over the entire colony, larger ones may develop in groups round the inoculum. These may reach a height of 7 mm. and like the smaller ones bear many short slender branches giving the synnemata a coralloid appearance. If the surface of the colony is examined under the microscope the globular heads of conidia are easily visible entirely covering the larger, but usually only partially covering the smaller synnemata. The mucus on the spores is not so conspicuous as it is when the fungus is growing under natural conditions, i.e. on the *Cordyceps* sclerotium, where each head is enveloped in a globule of mucus, but there is still sufficient to make them adhere, and form a head. Individual heads measure between 6 and 7  $\mu$ . The colony is brown in reverse.



Text-fig. 10. Conidiophores and conidia of *Tilachlidium brachiatum* (Batsch.) Petch.

*Tilachlidium brachiatum* (Batsch.) Petch, is a member of the Stilbaceae. As Petch (1945) has emphasized, *Tilachlidium* is not a coremial *Cephalosporium*, as there is a central core of agglutinated hyphae, the outer branches of which bear the conidia, instead of the tip of each hypha of the clava ending in a conidiophore. Petch found that the form known as *Isaria brachiata* (Batsch) Schum. was really a *Tilachlidium*, and named it *T. brachiatum* (Batsch.) Petch. He also included the type species *T. pinnatum* Preuss and *T. subulatum* A. L. Sm. under *T. brachiatum* as simple forms of the latter. It occurs on a variety of substrata, including leaves, roots, and decaying agarics, and is parasitic on *Stilbum tomentosum* and certain entomogenous fungi. Petch states that 'the clavae occur in clusters and are white to pale brown, simple or branched'. The whole clava is covered with *Cephalosporium* conidiophores, which are perpendicular to the main axis.



The conidia are described as 'cylindrical or narrow oval, hyaline, 2-7 (usually 3-6)  $\times$  0.75-1.5  $\mu$ '. These dimensions are different from those of the form on *Cordyceps aphodii*, but the dimensions appear to vary considerably within the species, for Petch gives the size of the conidia of the form on *Stilbum tomentosum* as 5-12  $\times$  2-4  $\mu$ , and of a specimen of *Isaria brachiata* on a leaf hopper as 2-4  $\times$  0.75-1.0  $\mu$ . Since these have all been included by Petch in this species, the form on *Cordyceps aphodii* has also been called *Tilachlidium brachiatum* (Batsch.) Petch.

#### DISCUSSION

In his monograph on the genus *Cordyceps*, Kobayasi (1941) regards the relationship between the conidial stage and the perfect stage as certain even if the conidia are merely produced on the perfect stroma or on a mycelium covering the endosclerotium. A relationship on these grounds was claimed by Tulasne (1853) for *Isaria farinosa* and *Cordyceps militaris*, although he did grow the fungus from part spores and Petch (1936), after examining his figures of conidiophores which develop from these colonies, considers that they are those of the true conidial stage which is a *Cephalosporium*, and not those of *Isaria farinosa* as Tulasne maintained.

In four species only of *Cordyceps* has the relationship between the conidial and the perfect stage been established in culture. *C. militaris* was shown by Petitt (1895) and by Petch (1936) to develop *Cephalosporium* conidiophores in culture; *Cordyceps clavulata*, for which Petitt (1895) obtained an asexual stage in cultures of hyphal bodies which corresponded with that formed under natural conditions, and has since been described by Petch (1933) as *Hirsutella lecaniicola* (Jaap) Petch; and Möller (1901) obtained *Cephalosporium* conidiophores from ascospore cultures of *Cordyceps rubra* and *C. submilitaris* (*C. martialis*). It has not been possible to obtain perithecia in culture from any of the spores of *C. aphodii*, but as spores from both of the suspected conidial stages produce a colony which is similar to that produced by the part spores, and they all develop the same type of conidia, it is very probable that they are all forms of the same fungus.

As far as I am aware, conidiophores similar to those of conidial stage A have never been described before, either as a conidial stage of *Cordyceps*, or in the Fungi Imperfecti. They bear a superficial resemblance to the bulbils produced by many genera of fungi, which, when they are not connected with a perfect stage, Hotson (1912, 1917) has described under the form genus *Papulaspora*. Although these bulbils are reproductive bodies, and consist of a mass of homogeneous or heterogeneous cells, one or all of which are capable of growth or germination, true conidia are never produced, nor anything approaching the complex structure of the head in stage A of this *Cordyceps*. *Papulaspora* has not been recorded on insects, but *Aegerita webberi*, which is similar in many respects, has been described by Fawcett (1910) on a scale insect and also by Petch (1926). This form resembles conidial stage A more closely than *Papulaspora*. Globose bodies arise from hyphae which grow out from the insect on the leaf; each one bears three to six setae and consists of a mass of globose or oval cells which arise in chains from the inflated apex of the stalk. However, there is still no suggestion of true conidia. Since it is contrary to the Rules of Botanical

Nomenclature to give a separate name to the conidial stage of a fungus when the perfect form is present, this stage must be described as *Cordyceps aphodii*, status conidialis A.

The second conidial stage has all the characters of the genus *Hymenostilbe* Petch, but the same ruling must apply, so that this stage must be described as *Cordyceps aphodii*, status conidialis B.

*C. aphodii* therefore has two conidial stages. This is a comparatively rare condition amongst species of *Cordyceps* and has only been recorded for *C. entomorrhiza*. For this species both *Hirsutella eleutheratorum* (Nees) Petch, and *Stilbella setiformis* (Vahl) Petch (Petch, 1933) have been described as conidial stages, the former arising from the same sclerotium and the latter as lateral branches of the stroma, but in neither case has proof of the relationship been obtained in culture.

Further work must be done to determine the conditions controlling infection of the host and the spread of the disease in the field before any conclusions can be drawn on the possible value of the fungus as a means of biological control of its host.

#### SUMMARY

1. The larva of the pasture cockchafer, *Aphodius howitti*, is the host for a new species of *Cordyceps*, *C. aphodii*.
2. The morphology of the fungus has been described in detail.
3. There are two conidial stages, and one of these, stage A, is undescribed; the other belongs to the genus *Hymenostilbe* Petch.
4. The fungus develops in the haemocoel of the host in the form of hyphal bodies which, after death takes place, form a mycelium of hyphae and gemmae.
5. The spores of all stages have been germinated and grown on artificial media. The same type of colony is produced by all types of spore, and all colonies develop conidial stage A only.
6. The *Cordyceps* is parasitized by two other species of fungi: (a) a member of the Discellaceae, and (b) *Tilachlidium brachiatum* (Batsch.) Petch. These have also been grown on artificial media and the colonies are unlike those of the *Cordyceps*.
7. Further work is necessary before the value of the fungus as a means of biological control of the host can be assessed.

#### *Cordyceps aphodii*

Mycelium in the endosclerotium white, consisting of hyphae about  $2.0\ \mu$  wide, and oval gemmae  $7.5\text{--}15.0\ \mu$  long and  $6.25\text{--}8.0\ \mu$  wide, which are solitary or several in a row, and terminal, intercalary or lateral on the hyphae.

Stromata caespitose, arising most frequently from the head of the sclerotium,  $1.0\text{--}1.4$  cm. long and  $1.0\text{--}1.5\ \mu$  wide, ochraceous brown.

Stem cylindrical, equal, glabrous, composed centrally of longitudinally running hyphae, and externally of hyphae arranged irregularly at right angles to the surface. Fertile part terminal but occasionally the tip of the stroma remains sterile; distinct from the stem, ovoid to cylindrical, but often distorted due to the unequal development of perithecia; 3–4 mm.

long and about 1.5 mm. broad, and the same colour as the stalk. The surface is slightly papillate due to the dark red-brown ostioles of the perithecia. Perithecia immersed in a loosely compacted layer between the central columella of longitudinally running hyphae and the outer compact layer. They are arranged obliquely to the main axis with upwardly curved necks, about  $357\mu$  long and  $110\mu$  broad. The wall is several cells thick and dark brown,  $6.75\mu$  wide. Asci  $200-225\mu$  long and  $3.8-5.0\mu$  broad. Cap  $8.0\mu$  broad and  $5.0\mu$  long. Ascospores  $90-151\mu$  long, articles oval, flattened at each end,  $5.5-7.5\mu$  long and  $1.25-2.0\mu$  broad.

*Status conidialis A*

Develops on the stromata and from the sclerote. Conidiophores on the ends of monopodially branched hyphae, consisting of an outer layer of buff-coloured tuberculate sterile hyphae which have short lateral branches on the convex side. They enclose a cavity filled with colourless conidia, which are budded off from a group of phialides at the apex of the stalk. Conidia oval with blunt ends,  $5.0-7.5\mu$  long and  $2.0-2.5\mu$  broad. Conidiophores about  $50-60\mu$  in diameter. Sterile hyphae about  $3.75\mu$  wide.

*Status conidialis B*

Develops on the sides of the stromata and consists of a palisade layer of phialides and conidia. Phialides are simple, smooth, slightly broader toward the apex, terminating in a fine sterigma,  $13.0\mu$  long and  $2-3\mu$  broad at their greatest width. Conidia slender, pip-shaped, hyaline smooth,  $5.0\mu$  long and  $1.25\mu$  wide. Spores dry.

*Cordyceps aphodii* n.sp.

Mycelio in endosclerotio, albo, hyphis circiter,  $2.0\mu$  latis et gemmis ovalibus  $7.5-15.0\mu$  longis  $6.25-8.0\mu$  latis composito; gemmis solitariis vel compluribus seriatim dispositis et terminaliter vel intercalariter vel lateraliter in hyphis.

Stromatibus caespitosis e capite sclerotii plerumque prorumpentibus,  $1.0-1.4$  cm. longis,  $1.0-1.5\mu$  latis ochraceo-brunneis.

Stipite cylindraceo, aequali, glabro, intus e hyphis composito longitudinaliter dispositis, extus e hyphis irregulariter et perpendiculariter in superficie sitis.

Parte fertili terminali sed stromatis apice nonnunquam sterili, stipite distincta, ex ovoidea cylindracea, sed saepe distorta ob crescendam inaequalem peritheciorum;  $3-4$  mm. longa circiter  $1.5$  mm. lata, eodem colore quo stipes. Superficie subpapillata ob ostiola rufo-brunnea peritheciorum.

Peritheciis immersis in strato laxo, compacto inter columellam centalem hypharum longitudinaliter dispositarum et stratum externale compactum; oblique ad axem principalem dispositis, collis sursum curvulis et circiter  $367\mu$  longis  $110\mu$  latis. Variete compluribus cellulis crasso, atro-brunneo,  $6.75\mu$  lato. Ascis  $200-225\mu$  longis  $3.8-5.0\mu$  latis. Capite  $8.0\mu$  lato  $5.0\mu$  longo. Ascoporis  $90-151\mu$  longis, articulis utrinque obtusis,  $5.5-7.5\mu$  longis  $1.25-2.0\mu$  latis.

*Status conidialis A*

In stromata et ex sclerotio oritur. Conidiophoris in apicibus hypharum monopodialiter furcatarum, strato externali hypharum tubercularum sterilium ochracearum compositis, quarum in latere convexo sunt breves ramuli laterales et quae cavositatem cingunt conidiis colore carentibus repletam, conidia velut gemmae gignuntur ex phialidibus in apice stipitis aggregatis. Conidiis ovalibus, apicibus obtusis  $5.0-7.5\mu$  longis  $2.0-2.5\mu$  latis. Conidiophoris circiter  $50-60\mu$  diameter. Hyphis sterilibus circiter  $3.75\mu$  latis.

*Status conidialis B*

In lateribus stromatum oritur, strato palisadeo phialisium et conidiorum compositus. Phialidibus simplicibus, teretibus, paullo latoribus ad apicem, sterigmate tenui terminatis,  $13.0\mu$  longis  $2.3\mu$  latis in parte latissima. Conidiis tenuibus ampullacea hyalinis, teretibus,  $5.0\mu$  longis  $1.25\mu$  latis, sporis siccis.

I would like to express my thanks to Professor J. S. Turner, in whose department this work was done, and to Associate-Professor E. McLennan, who has directed the investigation, for their continued help and advice.

I am indebted to Mr T. Petch for examining specimens of *Cordyceps aphodii* and confirming my diagnosis that it is a new species, and for naming the parasite *Tilachlidium brachiatum* (Batsch.) Petch. I would like to express my thanks for his kindness and advice.

Thanks are also due to Mr Twentymann of the Victorian Department of Agriculture, for supplying infected material, and for information regarding the habits of the larvae in the field.

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## EXPLANATION OF PLATES

PLATE XII, *Cordyceps*

- Fig. 1. Young stromata developing from the head of the larva.  $\times 3$ .
- Fig. 2. Stromata at a later stage, with conidial stage A, both on the stromata and on the surface of the sclerotium.  $\times 2$ .
- Fig. 3. Stromata developing from the body of a sterilized larva artificially inoculated with *Cordyceps aphodii*.  $\times 1\frac{1}{2}$ .
- Fig. 4. Stroma covered with conidiophores of conidial stage A.  $\times 10$ .
- Fig. 5. Layer of conidiophores of conidial stage A fallen from the stroma in a mass.  $\times 7$ .
- Fig. 6. Fertile clubs of *C. aphodii* projecting above the surface of the ground.  $\times 3$ .
- Fig. 7. Fertile clubs developed from sclerotes incubated in the laboratory.  $\times 3$ .

## PLATE XIII

- Fig. 1. Transverse section through fertile head of *Cordyceps aphodii*.  $\times 60$ .
- Fig. 2. Section through an infected larva of *A. howitti*, showing hyphal bodies clustered round the tracheal tubes.  $\times 200$ .
- Fig. 3. Section through an infected larva of *A. howitti*, showing hyphal bodies round the outer part of the gut wall, in the fat body, and round the muscles. The black spots in the fat body are protein granules which are heavily stained with haematoxylin.  $\times 170$ .
- Fig. 4. Longitudinal section through fertile head of *Cordyceps aphodii*.  $\times 20$ .

## PLATE XIV

- Fig. 1. Discellaceous form on *Cordyceps aphodii*—stipitate form. The yellow waxy areas are restricted to the tips of the clavae and to two patches near the base.  $\times 3\cdot5$ .
- Fig. 2. Discellaceous form on *C. aphodii*—open cup-shaped fruiting bodies on *Cordyceps* clava.  $\times 3\cdot5$ .
- Fig. 3. Discellaceous form—4 single spore cultures on potato-dextrose agar three weeks old.  $\times \frac{7}{5}$ .
- Fig. 4. *Tilachlidium brachiatum* (Batsch.) Petch on *Cordyceps aphodii* sclerotium.  $\times 5$ .

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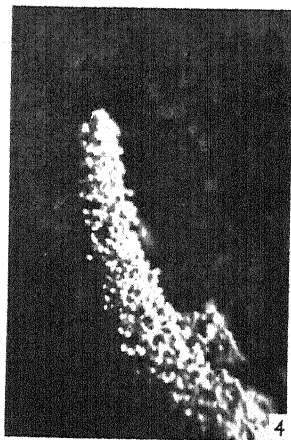
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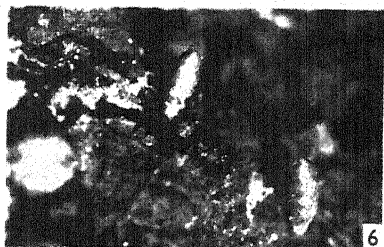
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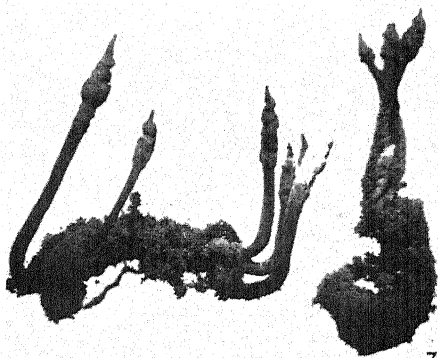
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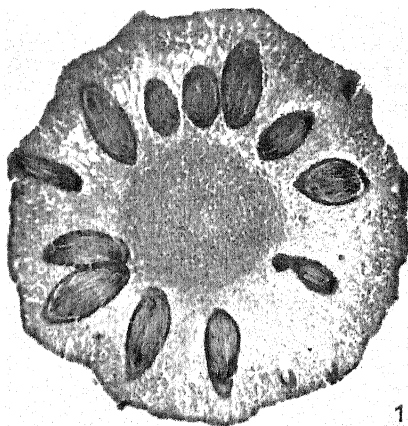
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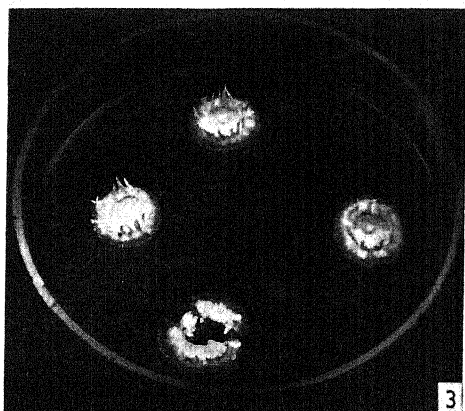
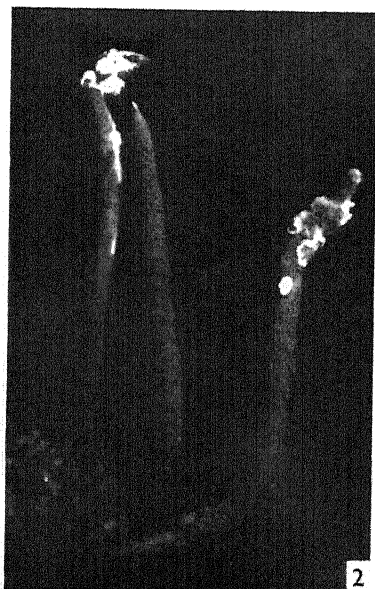


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# A COMPARATIVE STUDY OF PATHOGENIC ISOLATES OF *VERTICILLIUM*

By IVOR ISAAC

*Botany School, University of Cambridge, and East Malling Research Station*

(With Plates XV and XVI and 7 Text-figures)

## INTRODUCTION

Reinke and Berthold (1879), in their description of the causal organism of a wilt disease of potato, stated that the diseased tissue became black or dark brown due to a blackening of the hyphae of the pathogen, which had also become septate. The short 'cells' so formed increased considerably in breadth and became spheroid giving a torulose appearance to the mycelium. When neighbouring threads become contiguous, black cell masses of varying sizes and shapes and showing no longitudinal walls, appeared in the diseased tissue. These brown 'cells' and cellular masses constituted the resting mycelium for the over-wintering of the fungus and were called by the writers 'Dauermycelien' or 'Sklerotien'. They named the fungus *Verticillium albo-atrum*.

Klebahn (1913) isolated a form of *Verticillium* from dahlia which he considered differed sufficiently from the organism described by Reinke and Berthold to entitle it to distinct specific rank, and he named it *V. dahliae*. The principal point of difference, according to Klebahn, was that his isolate formed sclerotia which arose from irregular multilateral septation and budding of the 'cells' of a single hypha and not from the anastomosis of several hyphae.

Since 1913 the relationship between *V. albo-atrum* Reinke & Berthold, and *V. dahliae* Kleb., has been the subject of much discussion. Pethybridge (1919) isolated from the skin of a potato tuber a saprophytic species of *Verticillium* producing spherical, oval or pear-shaped chlamydospores in culture. He named this *V. nigrescens* and there appears to be no other mention of this fungus in the literature. In the course of my researches on *Verticillium* Wilt of sainfoin (Isaac, 1946) and hops (Isaac & Keyworth, 1948) I made a comparative study of a large range of isolates from these and other hosts and sources, and I propose to give evidence derived from this study that three distinct and constant fungus identities can be distinguished on morphological, physiological and phytopathogenic criteria.

A list of isolates used in the experiments to be described is given in Table 1. Throughout the text the microsclerotia, dark mycelium (resting mycelium or 'dauermycelien') and chlamydospore-forming types will be referred to as types M, D and C respectively.

Table 1. *Isolates used*

Designation of isolate	Host	Source of parent culture
M 1, M 1 var.	Sainfoin	Plant Breeding Institute, Cambridge
M 2	Antirrhinum	Botany School, Field Station, Cambridge
M 3	Chrysanthemum	Cheshunt Research Station
M 4	Tomato	Cheshunt Research Station
M 5	Potato	Botany School, Field Station, Cambridge
M 6	Sainfoin	Botany School, Field Station, Cambridge
M 7	Hop	East Malling Research Station
M 8	Raspberry	East Malling Research Station
M soil 1	Soil	Soil of Plant Breeding Institute, Cambridge
D 1 var.	Cucumber	Cheshunt Research Station
D 2 var.	Tomato	Cheshunt Research Station
D 3, D 3 var.	Cucumber	Rothamsted Experiment Station, Harpenden
D 4	Hop	Hop garden, Kent
D soil 1, D 4 var.	Soil	Soil of a Hop garden, Kent
C soil 1	Soil	Soil of Plant Breeding Institute, Cambridge
C soil 2	Soil	Soil of a Hop garden, Kent

M = microsclerotial type, D = dark mycelium type, C = chlamydospore type, Soil = isolation from soil, var. = hyaline variant arising in artificial culture.

## MORPHOLOGY OF ISOLATES

### RESTING MYCELIUM

The isolates used were those listed in Table 1. In each case single-spore cultures and cultures arising from a small portion of mycelium on Dox's agar were grown in 9 cm. Petri dishes, on Dox's, Czapek's, potato-extract, potato-dextrose, Brown's, 2.5% malt-extract, 5% malt-extract, oat-extract and 2% prune-extract agar media. Observations were also made on cultures derived from single spores in hanging drops of Dox's agar medium. All the cultures were exposed to normal daylight at room temperatures. From these observations it was concluded that each isolate could be grouped into one of the following types, according to the morphology of the resting mycelium:

*Type M (microsclerotial).* The fungi in this group constantly formed microsclerotia (length approximately 50–200  $\mu$ ) which gave a black appearance to the undersurface of the cultures on all media. Observations of the development of these resting bodies in hanging-drop cultures showed that after approximately five days' growth, contiguous hyphae became slightly swollen and divided by septa to form small 'cells' (Text-fig. 1*a*). After a further six days, new 'cells' had been budded off in all planes, forming a small, almost spherical mass (Text-fig. 1*b*). Soon, commencing from the centre of the mass, the walls of the 'cells' began to thicken and darken (Text-fig. 1*c*), eventually forming a microsclerotium (Text-fig. 1*d, e*). In some instances the 'cells' of an isolated thread multiplying by a process of budding gave rise to a microsclerotium. The best media for the development of the microsclerotial types were Dox's, Czapek's and potato-dextrose agars. In no instance were structures resembling the dark carbonized resting mycelium of type D observed in this group of cultures.

*Type D (dark mycelium).* The fungi in this group formed dark carbonized septate-resting mycelium which gave a black colour to the undersurface

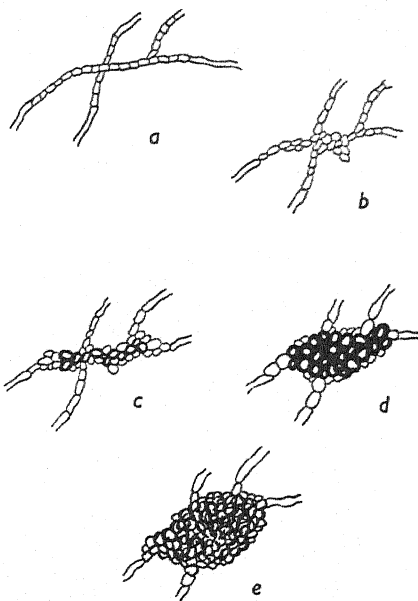
of the colonies. In a hanging-drop culture some hyphae were observed to become septate after eight days' growth (Text-fig. 2a), and within about four days the 'cells' so formed had become thickened to form black-resting mycelium (Text-fig. 2b). Occasionally torulose hyphae were formed by the individual 'cells' becoming swollen before the walls thickened.

No microsclerotia were observed in this group, but in Petri dish cultures on prune and potato-dextrose agars, after about three weeks' growth, black specks were seen. These were formed by the intertwining of the torulose hyphae giving rise to mycelial knots 100–500  $\mu$  in length (Pl. XV, fig. 1). In type M the resting bodies (microsclerotia) were formed from one hypha or more, but the mycelial knots of the second group were never formed from a single hypha, and while budding of 'cells' took place in the formation of microsclerotia no budding was observed in the production of mycelial knots. The latter were particularly well seen on plates where more than one colony had been grown (Pl. XV, fig. 2). It is suggested that Reinke and Berthold may have seen such structures and called them 'Sklerotien'. Berkeley, Madden and Willison (1931) and Keyworth (1942) observed these mycelial knots in their cultures of type D strains.

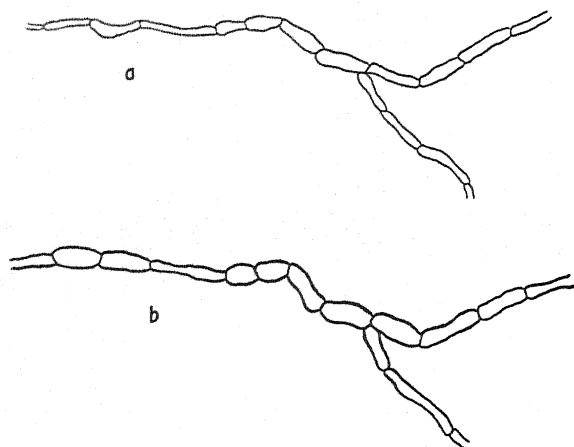
Potato-dextrose agar proved to be the best medium for the growth of this group of isolates.

*Hyaline variants.* Although the variants examined originated from hyaline saltants arising in Petri dish cultures of both microsclerotial and dark mycelium types, they all appeared identical in culture. Once the ability to produce black-resting bodies had been lost it was never recovered on any of the media used. The variants grew excellently on Dox's, Czapek's and potato-dextrose agar media, but conidia were produced only sparingly.

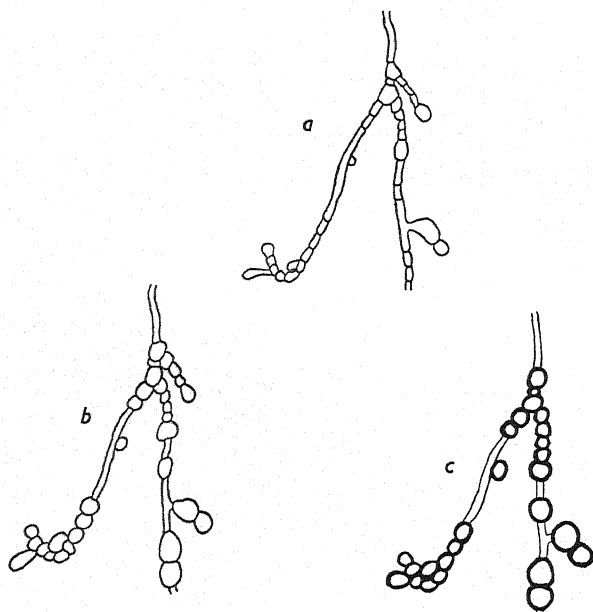
*Type C (chlamydospore).* The fungi in this group constantly formed spherical or pear-shaped chlamydospores (7–10  $\mu$  in diameter) giving a very dark brown to black colour to the undersurface. In a hanging-drop culture, septa appeared after about five days' growth, and these gave rise to 'cells' which within four days had become swollen and thickened to form chlamydospores (Text-fig. 3). These occurred in intercalary or terminal positions on the hyphae or were budded off the main hypha and had therefore a laterally sessile appearance. In old cultures 'cells' which did not give rise to chlamydospores became indistinct (Pl. XV, fig. 3).



Text-fig. 1. The development of a microsclerotium of M 1 from a pure culture on a hanging drop of Dox's agar. From a to e, after five, six, eight, ten and thirteen days' growth respectively. ( $\times 375$ .)



Text-fig. 2. The development of dark mycelium of D 3 from a pure culture on a hanging drop of Dox's agar. *a* and *b*, after eight and twelve days' growth respectively. ( $\times 375$ .)



Text-fig. 3. The development of chlamydospores of C soil 1 from a pure culture on a hanging drop of Dox's agar. From *a* to *c*, after five, seven and nine days respectively. ( $\times 375$ .)

#### DIMENSIONS OF CONIDIA

Van der Meer (1925) stated that her type M differed from her type D in spore size. I found no such constant difference: the dimensions of the conidia of the type M and type D and of the hyaline variants were  $3.5-10 \times 2.0-4.0 \mu$  (average  $6.5 \times 3.0 \mu$ ), and those of type C averaged  $7.0 \times 3.5 \mu$ .

## PHYSIOLOGY OF ISOLATES

In all the experiments dealing with the physiological reactions of *Verticillium* strains in culture,  $9 \times 2$  cm. Petri dishes were generally used with the media approximately 3 mm. deep. The stock cultures from which the inocula were taken were on Dox's and potato-dextrose agars. Using a sterilized cork-borer, blocks of inoculum 3 mm. in diameter were cut and transferred to the middle of the agar plates. Usually the growths of three to five replicate plates were averaged in each experiment.

Daily measurements of two diameters at right angles to each other were recorded to the nearest half-millimetre. When the colonies did not grow out circularly, as occasionally happened, the mean of the long and short diameters was recorded. But the diameters of colonies yield an imperfect measure of the actual growth of the fungi, for as Brown (1923) stated: 'the greatest objection to the lineal expansion is that it affords in many cases no indication whatever of the amount of mycelium in the fungal colony'. However, as a general measure of the relative growth of the *Verticillium* strains, the lineal method was convenient and provided a basis for comparing the influence of one temperature on different organisms or of different temperatures on the same organism.

## TEMPERATURE RELATIONS

There is considerable published work concerning the effect of temperature on the growth of *Verticillium* in culture from which it is clear that some confusion exists in the actual nomenclature adopted for the strains of fungi used. As Rudolph (1931) stated: 'Chaudhuri (1923) and Bewley (1922) have reported in detail the results of their cultural studies of *Verticillium albo-atrum* R. & B., as have Pethybridge (1916) and Vander Meer (1925). But in studying the data it is only too obvious that the first two workers were working with strains of the fungus which were not identical with that studied by the latter two.'

The aim of the present investigation was to determine and compare the temperature ranges of growth of each of the three mycelial forms of *Verticillium* described above.

The isolates used were: (1) type M (M 1 and M 7) with a hyaline variant (M var. 1); (2) type D (D 3 and D 4) and their variants (D 3 var. and D 4 var.); and (3) types C (C soil 1).

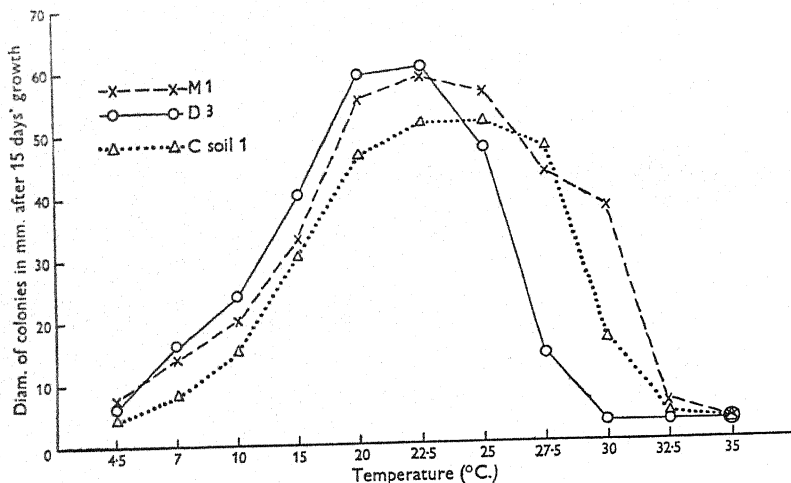
The determinations were made on three media, viz. normal Dox's, buffered Dox's (see p. 16) and potato-dextrose agars. The range of temperatures under which the cultures were incubated was 4.5, 7.0, 10.0, 15.0, 20.0, 22.5, 25.0, 27.5, 30.0, 32.5 and 35.0° C.

*Growth rates.* The results for one representative of each of the three mycelial types (M 1, D 3 and C soil 1) on potato-dextrose media are shown in Text-fig. 4. No appreciable deviations were recorded among the other isolates of each type and the variants closely resembled their cultures of origin in their temperature reactions. The results for each medium were also closely similar.



*Cultural morphology.* Wherever positive growth was recorded the morphology of the resulting mycelium was normal (i.e. true to the type of isolate employed). At 30° C., however, D<sub>3</sub>, D<sub>3</sub> var., D<sub>4</sub> and D<sub>4</sub> var. budded in a yeast-like manner and no hyphal growth occurred.

*Conclusions.* No differences were observed in the optimum temperature for the development of the types M and D and their variants; all showed best growth at 22·5° C., while type C differed from these by growing as well at 25·0 as at 22·5° C.



Text-fig. 4. Influence of temperature upon the growth of M<sub>1</sub>, D<sub>3</sub> and C soil 1 on Dox's medium.

At higher temperatures a marked difference was observed between type M, its variant and type C on the one hand, and type D and its variants on the other. The growth of the latter in contrast to the former declined very rapidly at temperatures above 25·0° C. At 30° C. the type M cultures and variant and type C form grew moderately well, whereas the types D and their variants merely produced small yeast-like colonies (Pl. XV, fig. 4). At 32·5° C. the M<sub>1</sub>, M<sub>1</sub> var., M<sub>7</sub> and C soil 1 cultures still showed a little growth, but no development occurred in the type D forms and variants. At 35·0° C. none of the isolates showed any growth. These results conform with those of Ludbrook (1933), who stated that his *V. albo-atrum* (type D) showed no growth at 30·0° C., while all his cultures of *V. dahliae* (type M) at this temperature showed some degree of development. The minimum temperature necessary for the growth of any of the strains appeared to be just below 4·5° C.

#### pH OF MEDIA

*Growth effect of isolates on pH.* To compare and contrast the effects of a type M (M<sub>1</sub>), a type D (D<sub>3</sub>) and a type C (C soil 1) form upon the pH of media, twelve cultures of each fungus were grown on each of three

media: (a) normal Dox's agar, (b) Dox's agar with 0.2% ammonium nitrate substituted for 0.2% sodium nitrate and (c) potato-extract agar. The cultures were incubated at 22.5° C. After six hours the pH of one plate of each fungus upon each medium was tested by the 'drop' method—using the B.D.H. indicators and comparing the results with the standards in Clark's Table (1923). After twelve hours and then on each subsequent day other plates were similarly examined.

This experiment brought out no differences between the three types, all of which rapidly produced a high degree of alkalinity in normal Dox's and potato-extract media, while with the ammonium medium they rapidly increased the degree of acidity. In every case a change in the pH value was noted before any sign of growth was observed and each fungus had an apparent effect upon the medium at a considerable distance from the colony.

By the end of three days the medium in every dish of Dox's and potato-extract agars was affected throughout its bulk, although the growth of the colony had been very slight. With modified Dox's agar plus ammonium nitrate all the medium was affected after a lapse of four days, although little development of the colony had occurred.

The maximum pH value induced by three types of *Verticillium* on Dox's or potato-extract agars was the same, viz. 8.4. On modified Dox's agar the lowest pH value reached was approximately 3.2 and at this degree of acidity growth was inhibited.

*Buffering of media.* Owing to the changes in the pH of the media used in the last experiment attempts were made to obtain a medium which would remain at a constant hydrogen-ion concentration throughout the growth of the fungi. Tests showed that by buffering Dox's medium with sodium and potassium phosphates a pH of 6.4 could be maintained during the growth of each type of isolate. This buffered medium is hereafter referred to as Buffered Dox's. It was also found that with potato-dextrose agar a pH of 6.4 was maintained for all isolates.

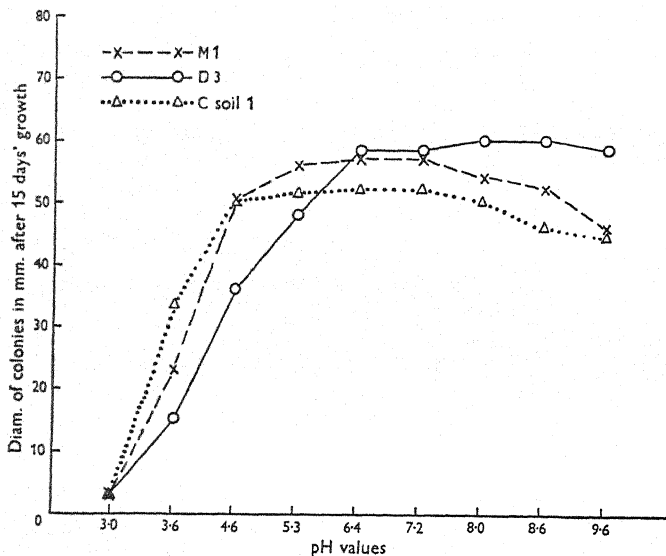
*Effect of varied initial pH on growth of isolates.* The same representatives of the three types of isolates as in the previous experiment (M<sub>1</sub>, M<sub>7</sub>, M<sub>1</sub> var., D<sub>3</sub>, D<sub>3</sub> var. D<sub>4</sub>, D<sub>4</sub> var. and C soil 1) were used on the same three media (Dox's, Buffered Dox's and potato-dextrose agars). The media were adjusted to the following range of pH values: 3.0, 3.6, 4.6, 5.3, 6.4, 7.2, 8.0, 8.6 and 9.6. For this purpose normal sodium hydroxide and normal hydrochloric acid were used for the Dox's medium and 2N sodium hydroxide and 2N hydrochloric acid for the two buffered media. Three plates of each medium at each pH were poured for each isolate and all were incubated at 22.5° C.

For the same reasons as given for the graph of temperature relationships the graph shown in Text-fig. 5 shows only the results for one microsclerotial (M<sub>1</sub>), one dark mycelium (D<sub>3</sub>) and one chlamydospore (C soil 1) type on potato-dextrose medium.

Where an old culture of M<sub>1</sub> was used the number of microsclerotia formed varied directly with the pH of the media: at 3.6 no sclerotia were produced, whereas at 8.0, 8.6 and 9.6 dense masses of these resting bodies appeared. Young cultures of M<sub>1</sub> produced sclerotia abundantly on each

plate where growth occurred. All the other fungi appeared normal at each pH where development took place.

This experiment brought out a marked difference between the type M, the variant M1 var., and the type C isolates, all of which showed best growth on all media between 5.3 and 7.2 and the type D isolates whose optimum pH for growth lay between 8.0 and 8.6. These results may be correlated to some extent with those of Verona and Ceccarelli (1935), whose figure 8.5, as the optimum pH for *V. albo-atrum*, corresponds very closely with my results for the type D forms, but 4.9 for *V. dahliae* is too low for the best growth of the type M isolates M1 and M7.



Text-fig. 5. Influence of pH of potato dextrose on the growth of M1, D3 and C soil 1.

The minimum pH value necessary for the growth of all the strains of *Verticillium* lay between 3.0 and 3.6, although at the latter value fairly good growth occurred. This does not agree with Haenseler's (1928) statement that *Verticillium* from egg-plant showed little or no growth below 4.0, or with Richardson's (1933) conclusion that *V. dahliae* grew at 2.3.

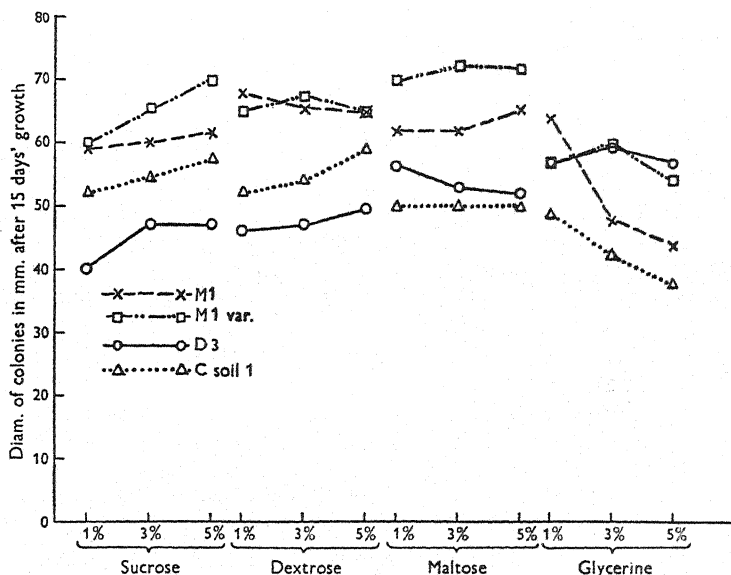
No differences were noted between the isolates in their power to modify the pH of media. As all the strains of *Verticillium* showed good development at the highest pH value used, it is not possible to state at what degree of alkalinity growth is inhibited.

#### VARIATION OF SOURCES OF CARBOHYDRATES

Representative strains of the three fungal types and their variants were grown on Dox's medium with sugar content varied by substituting the following sugars at 1, 3 and 5% for the normal 1.5% sucrose-sucrose, dextrose, maltose and glycerine—twelve variations of Dox's medium in all.

The isolates used were the same as were tested in the temperature-relations experiments (M<sub>1</sub>, M<sub>7</sub>, M<sub>1</sub> var., D<sub>3</sub>, D<sub>4</sub>, D<sub>3</sub> var., D<sub>4</sub> var., C soil 1) and four replicates of each of the twelve media were made for each isolate. The plates were incubated at 22.5° C.

*Growth rates.* As the variants of each type reacted similarly to each other but differently from the parent forms, one variant only (M<sub>1</sub> var.) is included in the following graphic summary (Text-fig. 6) of the results, together with one microsclerotial, one dark mycelium and one chlamydospore form.



Text-fig. 6. Influence of various carbohydrates upon the growth of M<sub>1</sub>, D<sub>3</sub> and C soil 1.

*Cultural morphology.* Except for M<sub>7</sub> which induced a reddish brown colour in the glycerine media all the isolates on all media grew true to type.

*Conclusions.* Types M and D showed differences in their reactions to the various sources of carbohydrate. The former grew well on media containing sucrose, dextrose and maltose, and poorly on glycerine, while the latter developed poorly on sucrose and dextrose media and showed maximum growth on glycerine. All the variants reacted similarly to each other; D<sub>3</sub> var. and D<sub>4</sub> var. in contrast to their parent types and, like M<sub>1</sub> var., grew well on sucrose and dextrose while maximum growth for the three was on maltose. Growth was fairly good on glycerine.

The chlamydospore form, as also the microsclerotial isolates, showed good growth on sucrose and dextrose media and poor development on glycerine. Growth was fairly good on maltose.

No general conclusions could be drawn from the variations of sugar concentration because the effect upon the growth of the fungi was not sufficiently consistent.

## VARIATION OF SOURCES OF NITROGEN

Representative strains of the three types of isolates and their variants were grown on Dox's medium containing maltose, which had proved suitable for each strain, instead of sucrose, and with the nitrogen content varied by substituting the following at 0.1, 0.5 and 1.0% for the normal 0.2% sodium nitrate—asparagine, peptone, sodium nitrate, ammonium sulphate and ammonium nitrate. It has been shown that unbuffered ammonium medium rapidly becomes too acid for the growth of *Verticillium*, so to counteract the fall in pH value a 1.0% ammonium sulphate medium was buffered with calcium carbonate (0.1, 0.5 and 1.0%) and as a comparison a 1.0% sodium nitrate medium was similarly buffered.

The isolates were the same as those used in the previous experiments and four plates were poured of each of the twenty-one media. All were incubated at 22.5° C.

*Growth rates.* In the graph (Text-fig. 7) showing the results, one type M, one type D, one variant and the type C isolate are included.

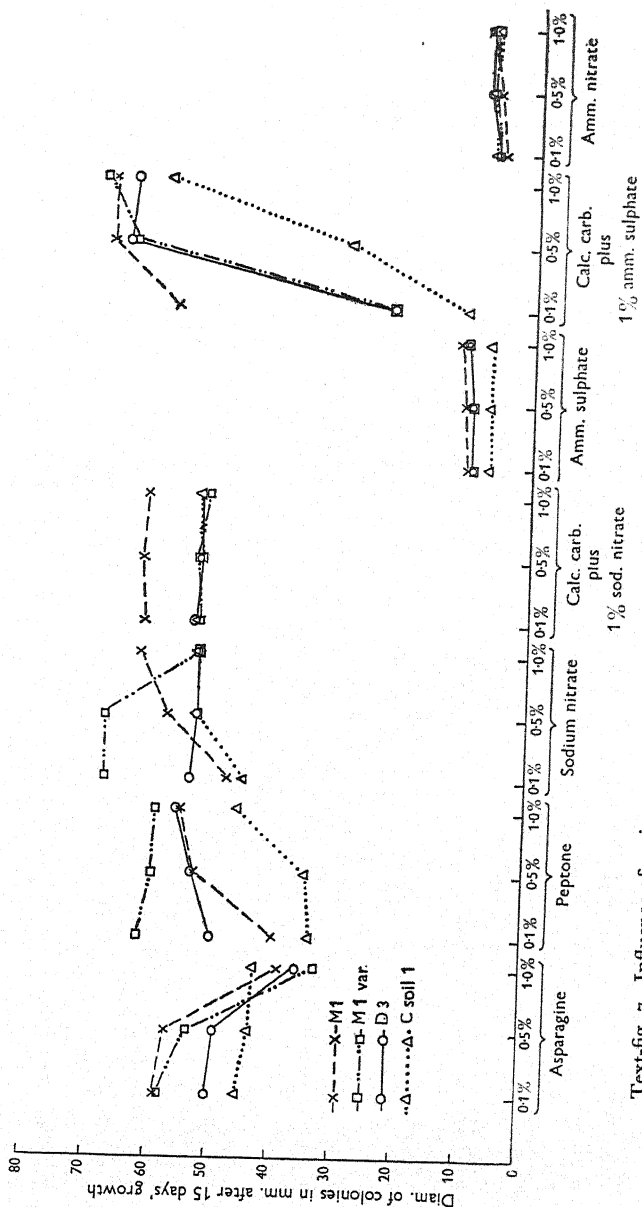
*Cultural morphology.* Asparagine. The type M and type C isolates appeared true to type on all asparagine media. The type D isolates, however, produced no black-resting mycelium on 1.0% asparagine, very little on 0.1%, but formed it abundantly on 0.5%. Except on 1.0%, where a small thick mat with a fawn coloured undersurface was formed, all the variants appeared normal.

Peptone. The M and C isolates grew true to type on all peptone media. The D isolate did not produce black-resting mycelium on 0.1%, very little on 0.5%, but formed it in abundance on 1.0%. Except on 0.1%, where a very thin mat with no aerial mycelium was formed, all the variants grew normally.

Sodium nitrate. All the isolates grew true to type on all the sodium nitrate media although the greatest tendency to form hyaline variants in the M and D types occurred here. The addition of calcium carbonate had no apparent effect on growth.

Ammonium sulphate and ammonium nitrate. On the unbuffered ammonium media of initial pH 5.3 all the colonies appeared as small abnormal, yeast-like clumps. No sporing occurred. Where 0.1% calcium carbonate had been added to the ammonium media the types M produced very thin white mats of irregular outline, with very few microsclerotia and no spores. The D isolates produced small thick mats with no black-resting mycelium and very few conidiophores. The variants produced small abnormal mats of mycelium with no spores. The chlamydospore form (C soil 1) isolate grew true to type except that little sporing occurred. Where 0.5 and 1.0% calcium carbonate had been added to the ammonium sulphate medium all the isolates grew true to type.

*Conclusions.* All the isolates were similar in showing maximum growth on media containing ammonium as a source of nitrogen buffered with calcium carbonate at 6.4. However, a slight difference between the types appeared on the sodium nitrate media, and increase in the sodium nitrate concentration caused an increased rate of growth of the microsclerotial and



Text-fig. 7. Influence of various sources of nitrogen upon the growth of M 1, M 1 var. D 3 and C soil 1.

chlamydospore forms but a decline of the dark mycelium type, greater in the case of the variants of both types.

On 0.1 and 0.5% asparagine the microsclerotial and dark mycelium types and their variants made good growth, but an increase to 1.0% resulted in slowing down development. On all the peptone media these types grew well while the chlamydospore type showed only fair growth on asparagine and peptone.

Although maximum growth of all the isolates occurred on buffered ammonium media where there was no stabilizing calcium carbonate, the plates rapidly reached a degree of acidity which inhibited further growth. Where ammonium nitrate was used the fall in pH value suggested that the fungi preferred the nitrogen of the ammonium ion to that of the nitrate even to the extent of creating a medium upon which it could make no further growth.

## PATHOGENICITY STUDIES

### TEMPERATURE RELATIONS

Edson and Shapovalov (1920), working on *Verticillium* Wilt of potato, suggested that there were two strains of the fungus, one found in the northern regions of the U.S.A. and the other in the southern area. The southern strain, which formed microsclerotia in culture, showed a better adaptation to higher temperatures and grew fairly well at 30° C., while the northern strain in which microsclerotia were 'practically absent' adapted itself more readily to lower temperatures and did not grow at 30° C. Bewley (1922) stated that with tomato the months of June, July and August were unfavourable to the rapid progress of the disease and he suggested that control could be effected by raising the temperature of the tomato-house above 15° C., but he did not differentiate between his isolates and simply referred to his organism as *Verticillium albo-atrum*. Later, Williams (1946), working in the same laboratory, presented experimental evidence indicating that infection of a variety of tomato by *V. albo-atrum* was checked by warm moist conditions, and that by *V. dahliae* somewhat less so. Low temperatures were also found to favour infection of cucumbers by *V. albo-atrum*. Ludbrook (1933), differentiating between the two types, reported that *V. dahliae* (microsclerotial type) induced disease symptoms in egg-plant at soil temperatures of 12–30° C. but not at 32° C., whereas *V. albo-atrum* (dark mycelium type) caused disease at 28° C. and below but not at 30° C., the air temperature in each case being between 19 and 23° C.

To determine the relation between temperature and the pathogenicity of the various *Verticillium* isolates studied in the present work one type of M (M1) and one type of D (D3) isolates were compared on sainfoin and tomato as host plants, and the experiments were carried out in four glass-houses with a mean temperature of 21.5, 25, 27 and 29° C. respectively.

*Sainfoin*. Five common sainfoin plants (Hunter strain) were placed in each glasshouse: four were wound-inoculated with M1 isolate and one (control) was wounded only. After approximately three weeks, the five plants in the hottest house flagged and died, but when examined they showed no trace of *Verticillium* disease, which suggested that sainfoin



normally cannot survive at high temperatures. The inoculated plants in all the other houses showed symptoms of Wilt after five to seven weeks, while the controls remained healthy. It is concluded, therefore, that the type M *Verticillium* is pathogenic to sainfoin at a temperature as high as 27° C.

*Tomato.* Parallel experiments were carried out with tomato plants inoculated with M1 and D3. Ten plants, singly in pots, were placed in each glasshouse immediately after being wound-inoculated just below ground level; four were inoculated with one type of *Verticillium*, four with the other type, while two, with wounds only, were kept as controls.

The plants inoculated with M1 in glasshouses 1, 2 and 3 showed wilt after five to six weeks and the fungus was reisolated in each case, while those in glasshouse 4 displayed no wilt symptoms and *Verticillium* was isolated from one plant only. Wilted plants from other houses recovered when placed in glasshouse 4, but when replaced in their original houses quickly wilted and died. The controls in each house remained healthy.

Where type D was used only those plants in glasshouse 1 wilted, and when these were transferred to hotter houses some recovery occurred, but on being returned to glasshouse 1 they again wilted. Some of the plants from glasshouses 2 and 3 when placed in glasshouse 1 quickly showed symptoms of wilt, but on being returned to their original houses they recovered. The plants from glasshouse 4 showed no wilt, even when placed in glasshouse 1, which suggests that the temperature in the fourth house was too high for the inoculations to be successful. In reisolation attempts the fungus was obtained from all the inoculated plants in glasshouses 1 and 2, from two in the third house and from none in the fourth. The controls in each house remained healthy.

These results show that the relation of *Verticillium* wilt of tomato to high temperature differs according to the type of *Verticillium* used. Plants inoculated with M1 and those with D3 wilted when kept in a house with a mean temperature of 21.5° C., but higher temperatures than this (25, 27 and 29° C.) had an inhibiting effect upon the development of symptoms by D3 in tomato, whilst M1 induced wilt at 25 and 27° C. While it may be suggested that these results correspond to some extent with the reports of Edson and Shapovalov (1920) and Ludbrook (1933) of a definite difference in the reactions of various strains of *Verticillium* to higher temperatures, they do not conform with Bewley's conclusion that *Verticillium* wilt of tomato does not occur at temperatures higher than 24° C. Bewley's statement may have been based on work with a strain similar to the type D3, because while the control measure which he suggested is quite effective with this type, plants infected by type M do not recover unless subjected to an indoor temperature exceeding 27° C.

#### CROSS-INOCULATIONS

Cross-inoculation experiments involving various host plants and the nineteen isolates were made to determine whether the strains in question could be classified on pathological criteria. Units of four to twelve plants of each host in pots or in the field were inoculated with pure cultures of the fungi by the following methods.

Wound-inoculation was used for each host. The fungi were inserted into wounds made in the wood of the main stem just below ground level, the inocula being cut from cultures on Dox's and potato-dextrose agar media. Recent 'reisolutions' of the fungi were used to obviate any failure to cause infection due to loss of pathogenicity by prolonged artificial culture. Controls with wounds only were kept for each set of experiments.

The hypodermic syringe method was used only for hop. A water suspension of conidia was inserted through a hypodermic needle into the wood of the hop stems, about three feet above ground level.

The experiments were conducted in the summer months, the plants being kept in pots in a cold-frame unless otherwise stated. All the attempts to reisolate the fungus from the wilted plants at the end of each series of experiments were successful, and the isolate in each case proved to be morphologically identical with the inoculum.

Sainfoin (common), antirrhinum, cucumber, lucerne (Canadian Grimm), tomato (Kondine Red), egg-plant, strawberry (Royal Sovereign Malling 40), potato (Eclipse) and chrysanthemum in pots, and hop (Fuggle) and salsify growing in the field, were inoculated with strains of *Verticillium*. Although positive results were obtained with all the host plants except lucerne, no differences were observed between the symptoms and rate of progression of Wilt induced by microsclerotial and dark mycelium forms in antirrhinum, cucumber, and chrysanthemum, so these plants have been omitted from Table 2 which summarizes the results.

The *Verticillium* type D isolates induced symptoms in salsify, egg-plant, tomato, strawberry, potato and hop sooner than the M types, but both were similar in their action on other host plants used. Usually the rate of progression of Wilt caused by the type D was very much greater than that caused by the type M isolates, particularly in strawberry.

All the types M and D isolates from hop were pathogenic to sainfoin, but the D isolates from cucumber and tomato failed to induce Wilt in this host.

The type C isolates were not pathogenic to sainfoin, strawberry and cucumber; in the susceptible hosts the rate of development of Wilt caused by these organisms was less rapid than that induced by the D and M forms.

All the strains produced similar symptoms of Wilt. Usually the first symptom was a loss of colour in the lower leaves which later became pale and desiccated and then fell; higher leaves progressively became affected until the whole plant was defoliated. However, antirrhinum always, and hop, potato and tomato occasionally, showed 'true-wilt', i.e. all the leaves becoming flaccid simultaneously accompanied by a loss of turgor in the shoots.

Except where 'true-wilt' occurred the woody tissue of diseased plants was discoloured owing to the presence of gum and mycelium in the vessels and to the brown staining of the vessel walls. The presence of the fungus usually stimulated the production of tyloses in vessels, e.g. in potato, tomato, hops and cucumber.

Table 2. *Pathogenicity tests of Verticillium isolates*

Inoculum*	Saintfoin		Salsify		Egg-plant		Tomato		Strawberry		Potato		Hop	
	Wilted†	Incubation (weeks)‡	Wilted†	Incubation (weeks)‡	Wilted†	Incubation (weeks)‡	Wilted†	Incubation (weeks)‡	Wilted†	Incubation (weeks)‡	Wilted†	Incubation (2 weeks)‡	Wilted†	Incubation (weeks)‡
M 1	12/12	3-5	4/5	4-6	5/5	5	4/4	4-5	3/4	10-12	8/10	3-5	12/12	3
M 1 var.	9/9	3-5	—	—	—	—	4/4	4-5	2/4	10-12	—	—	—	—
M 2	3/3	3-5	—	—	—	—	3/4	4-6	—	—	—	—	—	—
M 3	3/2	3-5	—	—	—	—	4/4	4-5	—	—	—	—	—	—
M 4	3/3	4	—	—	—	—	4/4	4-5	—	—	—	—	—	—
M 5	3/3	4	—	—	—	—	4/4	4-5	—	—	—	—	—	—
M 6	3/3	4	—	—	—	—	3/4	4-5	—	—	9/10	3-5	—	—
M 7	6/6	3-4	5/5	4-5	4/5	4-5	3/4	4-6	—	—	7/10	3-6	—	—
M 8	6/6	3	5/5	4-5	5/5	4	4/4	4	4/4	8-9	7/10	3-7	12/12	3
M soil 1	3/3	3-4	—	—	—	—	3/4	4-5	—	—	—	—	—	—
D 1 var.	0/12	—	—	—	—	—	4/4	3-4	—	—	—	—	—	—
D 2 var.	0/12	—	—	—	—	—	4/4	3-4	—	—	—	—	—	—
D 3	0/12	—	5/5	2-3	5/5	3	4/4	3-5	—	—	10/10	2-3	—	—
D 3 var.	0/12	—	—	—	—	—	4/4	3-4	—	—	—	—	—	—
D 4	12/12	3-5	5/5	2-3	5/5	2-3	4/4	3-4	7/8	4-7	10/10	2-3	12/12	2
D 4 var.	12/12	3-5	—	—	—	—	4/4	3-5	4/4	4-7	10/10	2-4	—	—
D soil 1	8/9	3-5	5/5	2-3	5/5	2-3	4/4	3-4	4/4	4-6	—	—	—	—
C soil 1	0/12	—	3/5	6	2/5	6	3/4	6-7	0/4	—	9/10	3-6	2/6	3-5
C soil 2	0/12	—	—	—	—	—	4/4	6-8	0/4	—	8/10	3-8	—	—

\* For list see Table 1.

† Numerator shows plants wilted, denominator, total plants inoculated.

‡ Number of weeks elapsing between inoculation and first symptoms of Wilt.

All controls in each series remained normal

## CONCLUSIONS AND DISCUSSION

The morphological differences between the three mycelial types of *Verticillium* in culture leading to their designation as microsclerotial, dark mycelium, and chlamydospore forms have been fully described in a foregoing section, and here it must be stressed that these differences have without exception remained constant throughout the present investigation, i.e. M type has never given rise to type D and vice versa. To these differences are now added those derived from physiological and pathological studies and summarized in Table 3.

Table 3. *Comparison of the three mycelial types*

	Type of resting mycelium				
	M	M var.	D	D var.	C
Optimum pH for growth in culture	5.3-7.2	5.3-7.2	8.0-8.6	8.0-8.6	5.3-7.2
Carbohydrate in culture	Best on sucrose and dextrose, very poor on glycerine	Best on maltose	Best on glycerine	Best on maltose	As type M
Growth in culture at 30° C.	Fairly good	Fairly good	Nil	Nil	Fairly good
Temperature and Wilt symptoms	Wilt up to 27° C.	—	Wilt at 21.5° C. but not at 25 and 27° C.	—	—
Pathogenicity	Less rapidly than type D on hop, potato, tomato and strawberry. All isolates pathogenic to sainfoin	As type M	See type M. Some isolates non-pathogenic to sainfoin	As type D	Less rapidly than types M and D. Non-pathogenic to sainfoin, cucumber and strawberry

If it is concluded from these data that we are dealing with three distinct organisms or groups of organisms the question arises which of them, if any, corresponds to the *V. albo-atrum* of Reinke and Berthold. I suggest that these workers were dealing with an organism identical with the type D isolates described above.

Reinke and Berthold, describing their fungus, stated that brown cellular 'Heaps', 'Zellhaufen', 'Dauermycelien', or 'Sklerotien', which never showed longitudinal walls, were formed when neighbouring hyphae came together, and maintained that these were not true tissue bodies or sclerotia. It is difficult to visualize a tissue body with only transverse walls.

Reinke and Berthold's drawing of their 'Dauermycelien' (Pl. XVI, fig. 5) suggests the resting mycelium of D<sub>3</sub> (Pl. XVI, fig. 6) rather than the microsclerotia of M<sub>1</sub> (Pl. XVI, fig. 7). In my type D isolates I observed, on prune and potato-dextrose agars (Pl. XV, fig. 2), black 'cell' masses or mycelial knots which were clearly seen to be formed by neighbouring hyphae becoming twisted about each other. The budding of the 'cells' of a single hypha or of a few hyphae, which is characteristic of the formation of sclerotia, has in no instance been observed in these isolates.

Klebahn (1913), Pethybridge (1916), Van der Meer (1925), Berkeley *et al.* (1931), Ludbrook (1933) and Van Beyma (1939-40) supported the

contention that *V. albo-atrum* R. & B. does not produce true sclerotia. Bewley (1922), Haenseler (1928), Van der Veen (1930), Presley (1941) and McKeen (1943) considered the sclerotia and resting mycelium producing fungi (i.e. types M and D) as one and the same organism, viz. *V. albo-atrum* R. & B.

Wollenweber (1929) preferred to call *V. albo-atrum* R. & B. a sclerotial form. The fungus producing dark carbonized resting mycelium, he said, was rare and should be considered as a variety of *V. albo-atrum* R. & B., which he called *V. albo-atrum* var. *caespitosum*.

Rudolph (1931) maintained that Reinke and Berthold were obviously familiar with true sclerotia, because in their review of Hallier's work (1878) they stated that the structure drawn by Hallier (Pl. XVI, fig. 8) 'was the black-brown Dauermycelien of *Verticillium*'. It must be admitted that the structures drawn by Hallier were undoubtedly sclerotia, but they are certainly different from the 'Dauermycelien' drawn by Reinke and Berthold, Hallier's drawing definitely showing a tissue-like structure with longitudinal walls, which Reinke and Berthold stated was absent in their organism.

Rudolph also reported that the Centraal Bureau voor Schimmelcultures at Baarn, Holland, issued to him as *V. albo-atrum* a fungus which on Czapek's agar formed microsclerotia. Berkeley *et al.* (1931), however, stated that they received three different cultures of *V. albo-atrum* from Baarn, Holland, all of which formed dark mycelium. Van Beyma (1939-40), publishing from this Institution, stated that the Centraal Bureau regarded *V. albo-atrum* as a form not producing microsclerotia.

Rudolph concluded that the fungus which produces only black carbonized hyphae and no sclerotia should be regarded merely as a variety, *V. albo-atrum* var. *tuberosum*. This latter is clearly synonymous with *V. albo-atrum* var. *caespitosum* Wollenw., and both, in my opinion, are simply the original *V. albo-atrum* R. & B.

If this conclusion is accepted another name must be assigned to the type M isolates used in this investigation and the writer suggests that these correspond very closely to *V. dahliae* Kleb. (Pl. XVI, fig. 9). Klebahn argued in favour of assigning specific rank to his fungus simply on its ability to produce microsclerotia as distinct from resting mycelium. Van der Meer (1925), Berkeley *et al.* (1931), Ludbrook (1933) and Van Beyma (1939-40) have supported his contention.

Table 3 provides ample grounds for dismissing the suggestion put forward by Bewley (1922), Haenseler (1928), McKeen (1943) and others that *V. albo-atrum* R. & B. and *V. dahliae* are one and the same organism. Whatever the rank accorded to them constant differences undoubtedly exist and we clearly have to deal with two organisms or groups of organisms of distinct identities. Are my types D and M then to be regarded as *V. albo-atrum* and a variety of *V. albo-atrum* respectively, or as distinct species? I was compelled for my research purposes tentatively to assign them different names, and in so doing was prompted not to add to present confusion and lengthy synonymy yet another name, unless compelled to do so on the well-established and generally accepted rules of fungus nomenclature.

Regarding type D ample data led to the conclusion already stated that this is the original *V. albo-atrum* R. & B. The remaining question was whether the difference between *V. albo-atrum* R. & B. and the type M entitled the latter to specific rank or merely to the rank of a variety of *V. albo-atrum*. If the former, then in my opinion it should be called *V. dahliae* Kleb., for Klebahn was the first to describe the distinctive structure of the microsclerotium. If the latter, then a new name must be given, e.g. *V. albo-atrum* var. *dahliae*.

In his examination of the criteria of fungus classification, Butler (1926) enumerated three main categories of distinction: morphological, biological, and a combination of both. Neither this author nor Cunningham (1926) favoured the distinction of species on biological criteria, but Cunningham regarded the division of species on such grounds as inevitable.

Dealing first with the morphological criteria, if by such Butler and Cunningham imply differences in the morphology of reproductive bodies, then the type M is not entitled to specific rank because no sexual reproductive bodies for *Verticillium* are yet known, and the D and M types have not been found to differ in the size of their conidia or conidiophores. But they have been found to differ consistently in the morphology of their resting bodies in culture, and I find it difficult to see why such a distinction should not rank at least as high as differences between asexual sporing structures.

It is true that morphologically indistinguishable hyaline saltants have appeared with each form in artificial culture, but even so, striking distinctions of a biological nature persisted, namely in their reactions to higher temperatures (30° C.) and in their optimum pH for growth. Thus a microsclerotial form and its variants can always be distinguished from the resting mycelium form and its variants on such cultural criteria.

An important physiological difference between types M and D lies in their respective maximum temperatures for growth (as pointed out above), and also in the maximum temperature at which Wilt is induced in their hosts: type M isolates can induce Wilt at 27° C., whereas type D isolates fail to cause wilt at 25° C.

It has been shown above that sometimes where temperature was equally favourable for the growth of either strain, different reactions of the fungi to differing host plants were observed (e.g. tomato, potato, hop and strawberry), type D inducing Wilt more rapidly than type M isolates. But whether pathogenicity should be taken into account in classifying these forms must be considered in the light of the fact that whereas all the type M isolates were pathogenic to sainfoin, of the type D isolates used only those from hop induced Wilt on this host. It is concluded that biologic forms of *V. albo-atrum* exist, but in my view this does not weaken the argument for considering the type M and D isolates as distinct species.

Whether the above differences, however, entitle type M to specific or merely to varietal rank depends on the definitions of these categories, and these are at present largely a matter of personal opinion and interpretation. Cunningham (1926), for example, has stated that 'a species is but a group of individuals separable from other groups placed in the same genus by the presence of certain characters whereby they may be recognized'. But it

may be argued that this definition can be applied equally well to the term 'variety'.

At present, therefore, the ultimate criterion of any system of classification we accept must be convenience (i.e. usefulness, simplicity and practicability). As Butler (1926) stated: 'if we admit that there is no such thing in nature as a pure invariable homogeneous species...convenience is often the best guide where to draw the line between different species'.

Upon this basis it can, therefore, be decided that type M should be given specific rank since it has never hitherto been regarded as a variety of type D, and that to regard it as such now would cause needless confusion and inconvenience to plant pathologists. The separation of these forms into distinct species is both useful and practicable, and it is therefore suggested that the microsclerotial form should be regarded as *V. dahliae* Kleb.

Finally, the status of the chlamydospore-producing fungus has to be considered. Pethybridge (1919) described a saprophytic form of *Verticillium* which produced chlamydospores (Pl. XVI, fig. 10), the development, shape and size of which appear very similar to those formed by C soil 1 and C soil 2 (Pl. XV, fig. 3). Pethybridge maintained that this strain differed sufficiently from *V. albo-atrum* to warrant specific rank and he named it *V. nigrescens*. *V. nigrescens* Pethybr. was originally described as a saprophytic form only, whereas my chlamydospore strains were parasitic to potato, tomato, antirrhinum, etc., but again this is not sufficient to justify a subdivision of Pethybridge's species. For similar reasons to those given above for distinguishing types D and M as distinct species, it is suggested that type C should also be accorded specific rank, and it is proposed to ascribe to it the name given by Pethybridge, viz. *V. nigrescens*.

The above conclusions are exhibited in summary form in Table 4.

Table 4.

Reference	Proposed nomenclature	Synonyms
Type D. Resting mycelium form	<i>Verticillium albo-atrum</i> Reinke & Berthold	<i>Verticillium albo-atrum</i> var. <i>caespitosum</i> Wollenw. <i>Verticillium albo-atrum</i> <i>tuberosum</i> Rudolph
Type M. Microsclerotial form	<i>Verticillium dahliae</i> Kleb.	<i>Verticillium albo-atrum</i> R. & B.—Bewley, Wollenweber, Rudolph, Presley, McKeen
Type C. Chlamydospore form	<i>Verticillium nigrescens</i> Pethybr.	—

## SUMMARY

The taxonomy of strains of *Verticillium*, forming microsclerotia, dark resting mycelium, and chlamydospores respectively, is discussed. In culture the optimum temperature for the growth of all the isolates was 22.5° C., but at 30° C. only the microsclerotial and chlamydospore strains developed. Optimum pH for the dark mycelium strains was 8.0–9.6, for the others 5.3–7.2. The microsclerotial and chlamydospore types grew well on sucrose, dextrose and maltose media and poorly on glycerine, while the dark mycelium strains developed well on glycerine, and poorly on sucrose and



dextrose media. The best source of nitrogen for all strains was the ammonium ion.

The microsclerotial form was pathogenic to sainfoin and tomato at 25 and 27° C., but at these temperatures the dark mycelium form did not induce Wilt. At normal temperatures the dark mycelium types were generally most rapidly pathogenic, but only those from hops induced Wilt in sainfoin.

Arguments are put forward for retaining distinct specific rank for the microsclerotial, dark mycelium, and chlamydospore strains, viz. *Verticillium dahliae* Kleb., *V. albo-atrum* Reinke & Berth. and *V. nigrescens* Pethybr. respectively.

This investigation was begun during the tenure of a scholarship awarded by the Ministry of Agriculture and Fisheries and the Agricultural Research Council. I wish to express my thanks to Prof. F. T. Brooks, who suggested the investigation, for his interest and advice throughout, and to the Agricultural Research Council and East Malling Research Station for means to continue the work in conjunction with research on *Verticillium* Wilt of hops under a special grant from the former. I am also much indebted to Dr R. V. Harris for advice and criticism in the preparation of this paper. The material has been used as part of a thesis presented for the degree of Ph.D. in the University of Cambridge.

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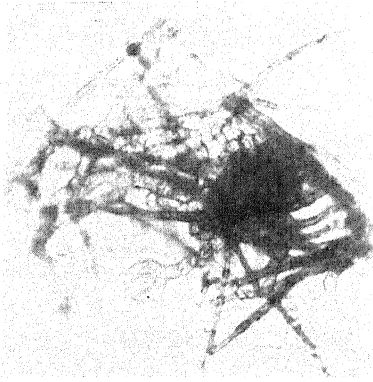


Fig. 1

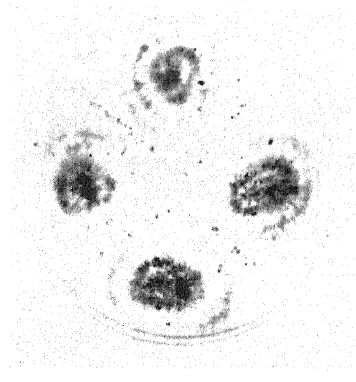


Fig. 2

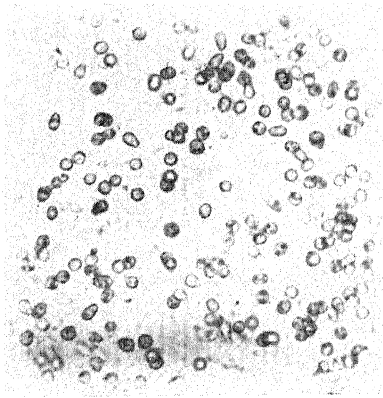


Fig. 3

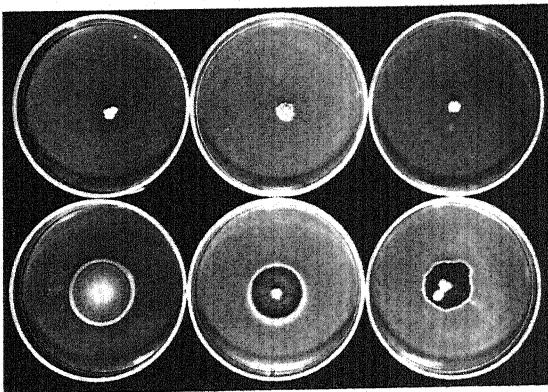


Fig. 4

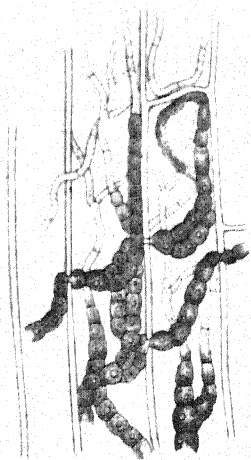


Fig. 5

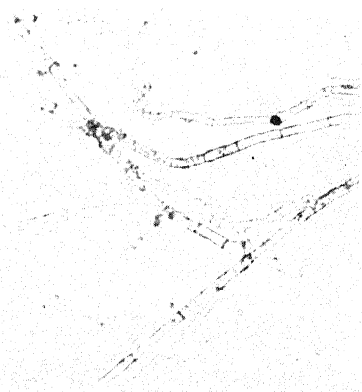


Fig. 6



Fig. 7

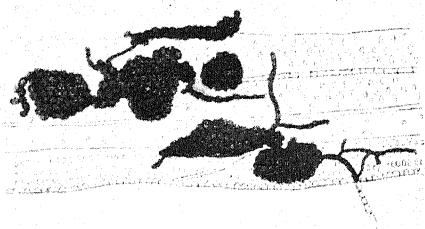


Fig. 8

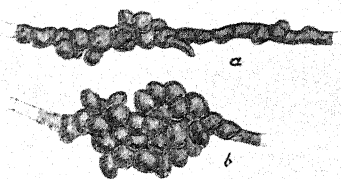


Fig. 9

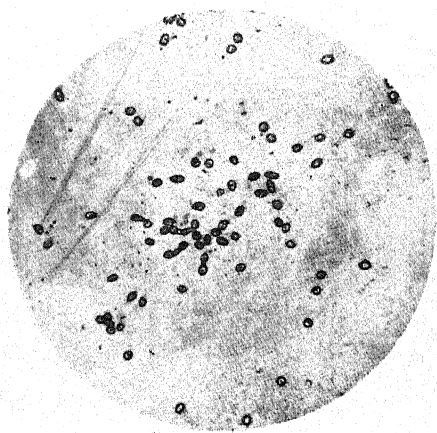


Fig. 10.

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## EXPLANATION OF PLATES

### PLATE XV

- Fig. 1. Mycelial knots (due to twisting about each other of torulose hyphae) of D 3 from a pure culture on prune-extract agar. ( $\times 332$ .)
- Fig. 2. Four pure cultures of D 3 on prune-extract agar. Note the small black resting bodies (mycelial knots) formed where the cultures meet.
- Fig. 3. Chlamydospores of C soil 1 from a pure culture on Dox's agar. Note mycelium almost invisible. ( $\times 332$ .)
- Fig. 4. D 3 (top row) and M 1 (bottom row) after fifteen days' growth at 30° C. on, left to right, potato dextrose, Dox's and Buffered Dox's media.

### PLATE XVI

- Fig. 5. Photographic reproduction of 'Dauermycelien' or 'Sklerotien' drawn by Reinke and Berthold. ( $\times 350$ .)
- Fig. 6. Dark mycelium of D 3 from a pure culture on Dox's agar. ( $\times 332$ .)
- Fig. 7. Microsclerotia of M 1 from a pure culture on Dox's agar. ( $\times 332$ .)
- Fig. 8. Photographic reproduction of sclerotia-like body drawn by Hallier.
- Fig. 9. Photographic reproduction of sclerotia-like body drawn by Klebahn. ( $\times 516$ .)
- Fig. 10. Reproduction of photograph of chlamydospores of *Verticillium nigrescens* Pethybr. ( $\times 310$ .)

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## STACHYBOTRYS DICHROA GROVE

By G. R. BISBY AND M. B. ELLIS

*Commonwealth Mycological Institute, Kew*

(With 2 Text-figures)

In 1943 the senior author concluded that *Stachybotrys dichroa* Grove (1886) was young *S. atra* Corda. Only two collections were then known, and the spores and phialides showed much resemblance to those of the common and rather variable *S. atra*. However, recent collections have now demonstrated that *S. dichroa* is evidently not uncommon on old herbaceous stems, particularly in damp areas, and that it is clearly distinct from *S. atra*, which occurs in soil and from soil passes to damp cloth, paper, and other substrata. The following collections of *S. dichroa* have been examined:

On dead thistle stem [*Cirsium palustre*], Bradnock Hayes, near Little Sutton, 10 October 1885; ex type collection in Herb. W. B. Grove, Birmingham University (slide filed as Herb. I.M.I. 18,006).

On dead stems of *Epilobium [hirsutum]*, Hebden Bridge, 30 June 1900, ex Herb. C. Crossland in Herb. Kew (slide filed as Herb. I.M.I. 19,217).

On dead stems of *Acanthus*, Lambourne Hill, Perranzabuloe, West Cornwall, 27 August 1944, F. Rilstone, No. 4445 (Herb. I.M.I. No. 555).

On dead stems of *Filipendula ulmaria*, Wheatfen Broad, near Surlingham, Norfolk, 9 June 1946, E. A. and M. B. Ellis (Herb. I.M.I. No. 5608a).

On dead umbelliferous stems, Thornton-le-Dale, Yorkshire, 12 April 1947, J. Webster, University College Hull Herb. No. 100 (Herb. I.M.I. No. 17,592).

On dead stems of *Angelica sylvestris*, grounds of University College, Exeter, 14 September 1947, J. Webster (Herb. I.M.I. No. 17,506).

Very thin 'Necol' mounts show that, in nature, *Stachybotrys dichroa* produces little or no superficial mycelium; narrow hyphae, 1-3  $\mu$  in diameter, are immersed in the substratum and only the upright hyaline conidiophores with their black shining heads of spores appear above the surface. The conidiophores arise close to one another and, as in *S. atra*, the slimy spore masses occasionally coalesce.

The phialophores are simple, septate (2-7 septa), straight or slightly curved, smooth-walled, hyaline and average 157  $\mu$  (range 86-270  $\mu$ ) long; each arises from a bulbous base, 12.2 (8-20)  $\mu$  in diameter, and tapers from 8.5 (5-11)  $\mu$  immediately above the bulb to 4.4 (3.5-7)  $\mu$  below the apex, then swells again to form a small head 6.6 (5-9)  $\mu$  in diameter. The swollen apex bears a single ring of five to six smooth-walled, hyaline phialides, 11.9 (9-17)  $\mu$  long and 4.4 (3.5-6)  $\mu$  in diameter at their widest points; they are almost cylindrical, rounded at the apex and are often slightly narrower at the base, but never obovate or pyriform as in *S. atra*.

The conidia are produced singly and successively in slime at the apex of the phialide and form black heads of spores 38 (20-90)  $\mu$  in diameter. When

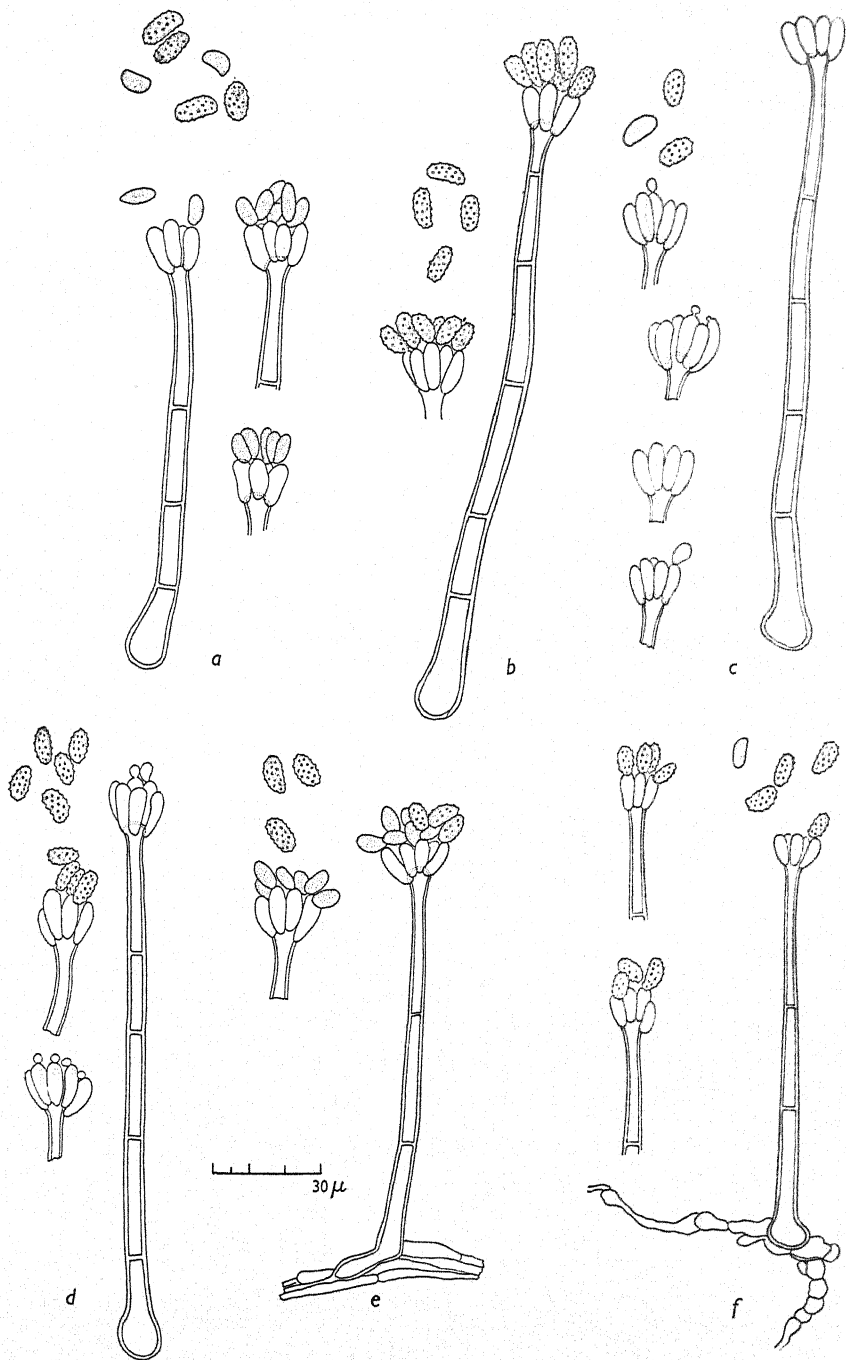


Fig. 1. *Stachybotrys dichroa*: phialophores (conidiophores), phialides, and conidia,  $\times 500$ . *a*, ex type collection (I.M.I. 18,006). *b*, I.M.I. 19,217 ex Herb. Crossland. *c*, I.M.I. 5608*a* on *Filipendula*. *d*, I.M.I. 17,506 on *Angelica*. *e*, *ibid.*, from culture after 19 days on P.D.A. *f*, I.M.I. 17,592 from culture after 19 days on P.D.A.

young the conidia are smooth-walled, pale olivaceous brown, ovoid or almost cylindrical, sometimes slightly curved, obtuse at the apex and often obliquely attenuated at the base; as they mature they become dark, finally almost black, and rough-walled. Mature conidia are  $9.8$  ( $8-12$ )  $\mu$  long with a maximum diameter of  $4.9$  ( $4-6$ )  $\mu$ . In fresh collections two large guttulæ may be seen in the conidia, but these disappear with age.

Culturally *S. dichroa* and *S. atra* are quite distinct, colonies of the former being a bright orange colour in reverse on potato dextrose agar, in contrast to the dark olive colour usually shown by *S. atra*. Although only six collections of *S. dichroa* have so far been studied (two of them also in culture), their uniformity indicates that the species is distinctive and easily recognized. It is curious that Grove's species should have remained almost unknown for so long.

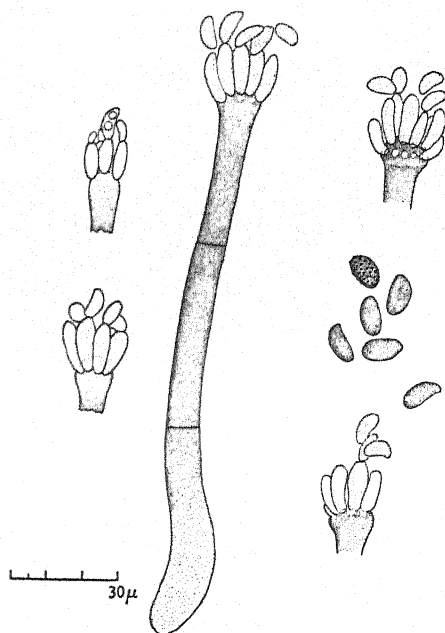


Fig. 2. *Stachybotrys* sp., I.M.I. 16,185 on *Oenanthe*, Guernsey,  $\times 500$ .

There is another, apparently so far undescribed, species of *Stachybotrys* with simple phialophores, which also occurs on dead herbaceous stems. Only one collection has been made: I.M.I. No. 16,185, on dead stems of *Oenanthe crocata*, La Bouvée, Guernsey, C. Isl., 19 June 1947, E. A. and M. B. Ellis. A description and figure of this fungus are appended, and it is being held for the time being as a taxonomically distinct species, but pending the receipt and examination of further collections is not being named and described as new.

Mycelium hyaline, septate, branched,  $1-3 \mu$  in diameter, embedded in the substratum. Upright phialophores simple,  $1-2$  septate, straight or slightly curved, smooth-walled, smoky grey to black,  $145$  ( $120-180$ )  $\mu$  long;



base paler and somewhat swollen but not markedly bulbous,  $12.3 (11-14) \mu$  in diameter, tapering above to  $6.9 (6-9) \mu$ , then swelling again to  $10.6 (9-12) \mu$ . The phialides are borne in one or two rings around the swollen apex; they are smooth-walled, hyaline at first, later smoky grey to black, cylindrical to obovate, often truncate at the apex,  $13.7 (11-16) \mu$  long and with a maximum diameter of  $5 (4-6) \mu$ .

The conidia are somewhat kidney-shaped, especially when young, rounded at the apex and sometimes obliquely attenuated at the base, and remain smooth for a long time; even when they had turned almost black, very few rough-walled conidia were seen; length  $10.1 (9-11) \mu$ ; maximum diameter  $6.2 (4.5-8) \mu$ .

The heads of conidia do not slime down in this species to the extent that they do in *Stachybotrys dichroa* and *S. atra*; partial chains of conidia are frequent.

In cultures on potato-dextrose agar at room temperature the colonies in reverse have a pinkish orange colour, similar to that of *S. dichroa*. The rate of growth is about half that of *S. dichroa* and the 'standard' culture of *S. atra*.

A word should be added regarding *S. subsimplex* Cooke, which Bisby (1943) thought might be the same as *Memmoniella echinata* (Riv.) Galloway. Zuck (1946) later found that *M. echinata* may sometimes produce spores in slimy heads rather than the usual chains, which strengthens the view that the two so-called species may be identical. As Zuck remarks, this leaves the name *Stachybotrys subsimplex* as doubtful, unless it refers to the 'Stachybotrys phase' of *Memmoniella echinata*. The latter proves to be a common fungus, as Bisby (1945) also reported. Incidentally, Zuck's observations throw doubt on the distinction between *Stachybotrys* and *Memmoniella*. The genus *Gliobotrys*, to which von Höhnelt referred *Stachybotrys dichroa*, still seems superfluous to us, though the type species, *Gliobotrys alboviridis* Höhnelt, requires restudy.

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ON *APHANOMYCOPSIS BACILLARIACEARUM*  
SCHERFFEL, *A. DESMIDIELLA* N.SP., AND  
*ANCYLISTES* SPP. IN GREAT BRITAIN

By HILDA M. CANTER

*The Freshwater Biological Laboratory, Wray Castle, Ambleside and  
Department of Botany, Birkbeck College, University of London*

(With 6 Text-figures)

I. *APHANOMYCOPSIS BACILLARIACEARUM* SCHERFFEL

*Aphanomycopsis* is at present represented by a single species *A. bacillariacearum*, described by Scherffel (1925). Sparrow later described specimens from America (1933) and recorded it from England (1936).

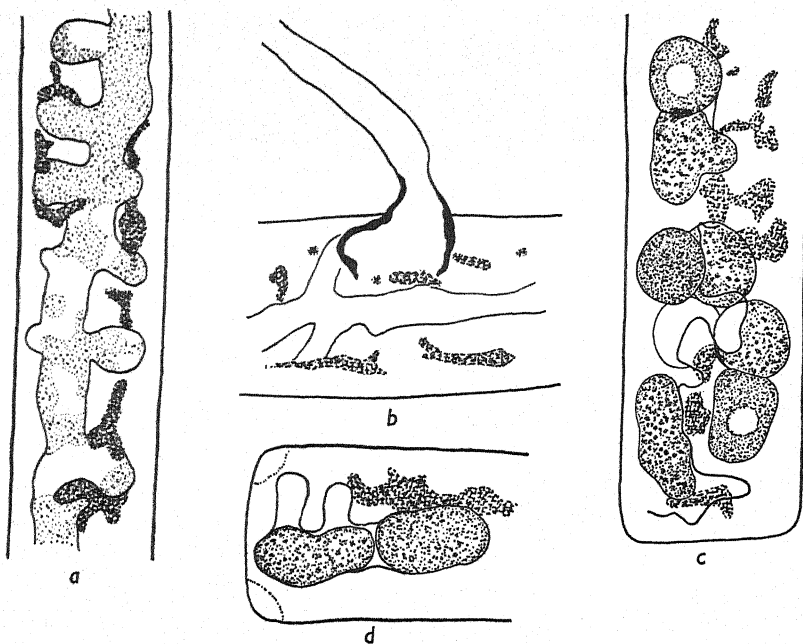


Fig. 1. *Aphanomycopsis bacillariacearum*. *a*, immature tubular thallus. *b*, empty sporangium, with exit tube which is thickened at the base, to form the so-called spreading apparatus. *c*, *d*, resting spores. All  $\times 500$ .

A few specimens clearly belonging to this species were found parasitizing *Pinnularia* sp. from Blelham Bog, near Wray Castle in January 1947. The sporangium consists of a long non-septate tube  $150\mu$  long,  $13\mu$  in diameter, with short lateral branches (Fig. 1*a*). The exit tube, up to

75  $\mu$  long by 9  $\mu$  in diameter, is considerably thickened and inflated at the base (Fig. 1*b*) to form the so-called spreading apparatus concerned with separating the valves of the diatom cell. In one specimen the exit tube had bifurcated outside the host cell. The spherical primary zoospore cysts, grouped around the mouth of the exit tube, are 10–12  $\mu$  in diameter and from them emerge the secondary biflagellate swimmers. Young stages in development of the resting spores only were seen (Fig. 1*c*, *d*). There is no sexual process and the resting spores are formed from contracted portions of the protoplasm.

## II. *Aphanomycopsis desmidiella* n.sp.

*A. bacillariacearum* appears to be limited to diatoms as do the other freshwater representatives of the Ectrogellaceae. What is considered to be a new species of *Aphanomycopsis* was found parasitizing a desmid (*Netrium digitus* (Ehrenb.) Itzigsch & Rothe) in a Sphagnum pool at Batemanfold, Lancashire, during late September 1946. An account of its life history follows.

The encysted zoospore germinates on the surface of the host cell producing a tube which penetrates the wall and forms within, a tubular swelling (Fig. 2*c*). This elongates, branches and finally forms a tubular non-septate contorted thallus 5–13  $\mu$  in diameter, filling the algal cell. The original zoospore case persists on the outside of the host wall and the first-formed portion of the thallus immediately within the wall often becomes swollen and may appear to be slightly thickened (Fig. 2*h*). By these characters it is often easy to find the original place of infection and to determine the number of individual thalli present (rarely more than two) in a single cell. The fungal protoplasm contains numerous highly refractive globules surrounding a central vacuole (Fig. 2*d*). One or two exit tubes push through the algal wall and grow into the surrounding water. They usually emerge from the ends of the *Netrium* cell (Fig. 3*a*), but sometimes they may push through the lateral wall. The exit tube is equally cylindrical throughout its length (5  $\mu$  in diameter) and varies from 10–185  $\mu$  long (the majority are 50–100  $\mu$  long). Growth in length of the exit tube is rapid; one observed under a cover-slip over a period of two and a half hours showed the following elongation in successive half hours: 15, 23, 16, 9, 7, 6  $\mu$ .

The actual cleavage of the protoplasm into the primary zoospores and their escape was never observed, but two specimens were seen in which they had recently emerged. It is evident that they do not swarm but form a motionless mass at the mouth of the exit tube. The encysted primary zoospores are spherical, 6–7.6  $\mu$  in diameter with granular protoplasm and a few refractive globules (Fig. 2*e*). The number of cysts formed may be up to sixty. After about an hour the naked secondary swimmer emerges (Fig. 2*g*). A few minutes after emergence, the flagella appear and the zoospore after oscillating for some time swims away. Its movement is smooth-gliding with sudden stops and changes in direction. The zoospores are somewhat bean shaped  $8 \times 7 \mu$  with two vacuoles, a small mass of refractive globules and two flagella (Fig. 2*a*); the longer posterior flagellum is dragged behind, while the shorter more active one is anterior.

No evidence of sexuality has been found in the development of the resting spore. As Scherffel described for *Aphanomycopsis bacillariacearum* it

appears to be developed from a rounded portion of the thallus protoplasm; from three to seventeen may occur lying loose in the expanded thallus wall. The spore is spherical to oval, 9–20  $\mu$  in diameter, with a thin wall and dense contents when young (Fig. 3c). As the spore matures, the wall thickens and becomes two-layered. The outer is highly refractive and on

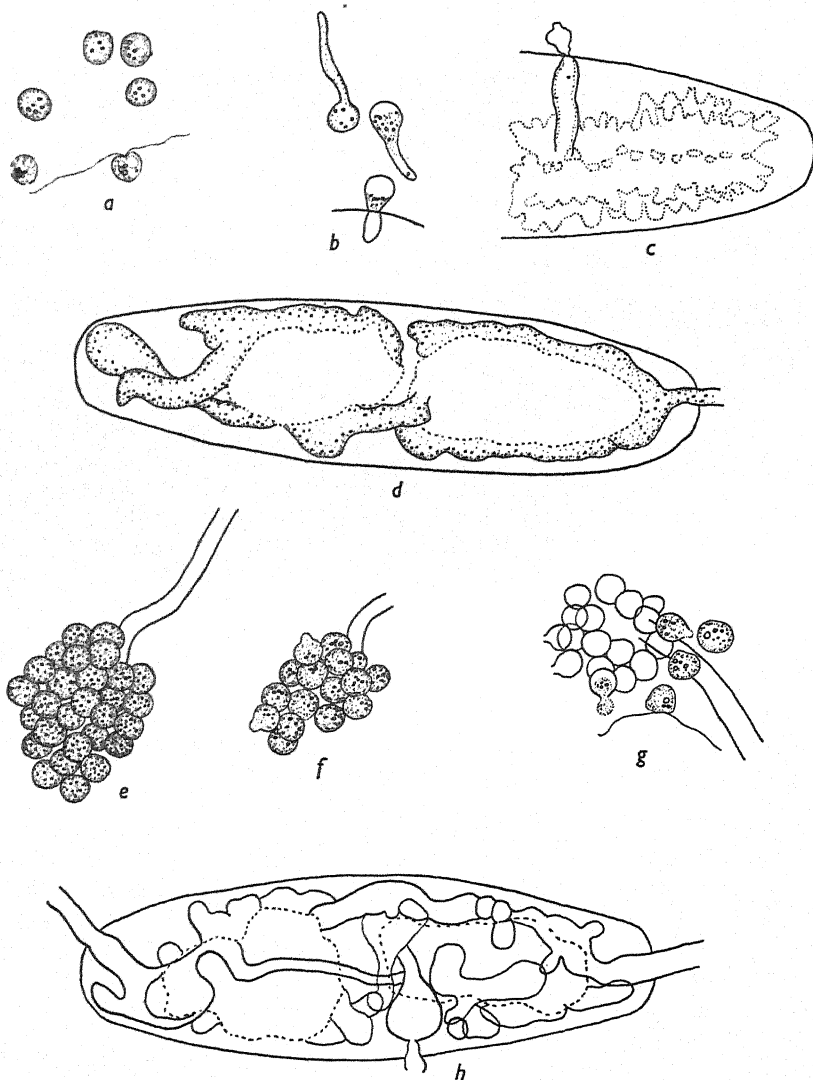


Fig. 2. *Aphanomycopsis desmidiella*. a, secondary zoospores. b, young stages in germination of zoospores; the two uppermost have germinated away from the algal cell. c, empty shrivelled zoospore case on algal wall, young unbranched tubular thallus inside. d, thallus with developing discharge tube. e, encysted primary zoospores. f, encysted primary zoospores, two with a papilla. g, empty primary zoospore cysts; emerging and free secondary zoospores. h, empty sporangium, swelling of thallus immediately beneath empty zoospore case clearly visible. b, c, h,  $\times 525$ ; a, d–g,  $\times 500$ .

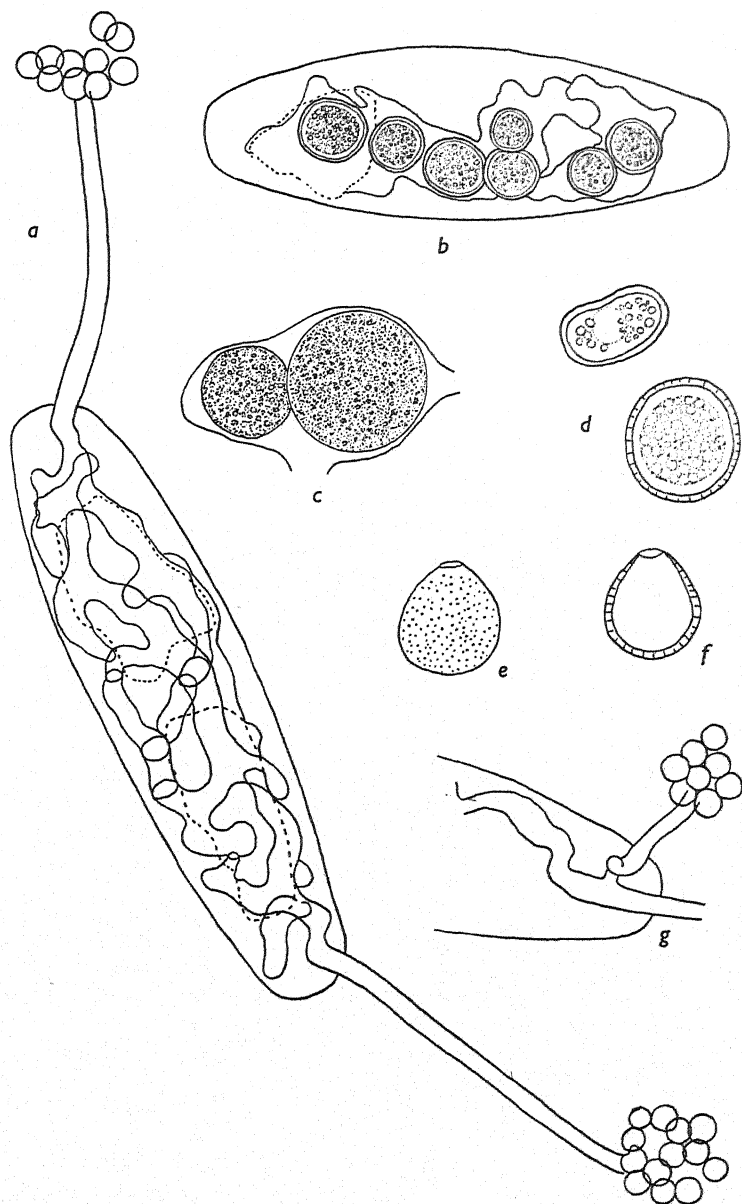


Fig. 3. *Aphanomycopsis desmidiella*. a, empty sporangium, two discharge tubes with attached, empty primary zoospore cysts. b, mature resting spores lying loosely in expanded thallus wall. c, two young resting spores with thin wall and dense contents. d, oval immature resting spores with two-layered wall and central globular contents. e, f, empty resting spore (e) in surface view, (f), in optical section showing the streak-like form of the surface dots through the outer wall. g, two dehiscence tubes emerging close together from the same thallus. a,  $\times 525$ ; b, g,  $\times 500$ ; e-f,  $\times 1050$ .

a dehiscence pore is seen to be covered with numerous dots (Fig. 3e). These markings in optical section appear to penetrate the outer wall (Fig. 3f); whether they are pores or more highly refractive thickening bands is unknown. The inner wall is hyaline and encloses the central content which consists of numerous refractive globules (Fig. 3d). How the resting spore germinates remains unknown. One empty resting spore with a dehiscence pore is shown in Fig. 3e, f. After treatment with chlor-zinc-iodide the thallus, discharge tube and resting spore wall stain pink; the zoospore case gives no reaction.

Because of the striking difference in the host it does not seem advisable to suggest that the present species is identical with *A. bacillariacearum*, although morphologically it differs from that species in very minor characters, notably in the size of the secondary swimmers, in the absence of thickening in the exit tube where it penetrates the host wall and in the sculpturing of the outer membrane of the resting spore. The binomial *A. desmidiella* is proposed for this parasite.

***Aphanomycopsis desmidiella* n.sp.**

Thallus endobiotic, branched, holocarpic, non-septate, forming a single zoosporangium. Zoosporangium  $5-13\mu$  in diameter, dehiscing by one to two exit tubes ( $10-185\mu$  long  $\times 5\mu$  in diameter). Primary zoospores (probably non-flagellate), forming ten to sixty spherical cysts ( $6-8\mu$  in diameter) at the mouth of the exit tube. Secondary zoospores ( $8 \times 7\mu$ ) kidney-shaped with two lateral flagella. Resting spores endobiotic, spherical to subspherical,  $9-20\mu$  in diameter, with a thick colourless wall and containing numerous small refractive globules; germination not observed.

Parasitic in *Netrium digitus* (Ehrenb.) Itzigisch & Rothe in Batemanfold, Lancashire, England.

*Aphanomycopsis desmidiella* sp.nov.

Thallus endobioticus, holocarpicus, ramosus, aseptatus, zoosporangium singulum generans. Zoosporangium  $5-13\mu$  diam., per 1-2 tubulos ( $10-185\mu$  longos  $5\mu$  latos) dehiscens. Zoosporae primariae (verisimiliter non-flagellatae) 10-60 cystos sphaericos ( $6-8\mu$  diam.) ad ostiolum tubuli efficientes. Zoosporae secundariae ( $8 \times 7\mu$ ) reniformes, flagellis lateralibus binis. Sporae perdurantes endobioticae, sphaericae vel subsphaericae,  $9-20\mu$  diam., tunica crassa hyalina, globulos refractivos numerosos parvos continentes. Germinatio non visa.

Hab. Parasiticus in *Netrio digito* (Ehrenb.) Itzigisch et Rothe, Bateman fold, Lancashire, Anglia.

Certain other incompletely described fungi found in desmids may be referable to this genus or even to *Aphanomycopsis desmidiella*. It is probable that a species of *Aphanomycopsis* caused Archer (1860) to claim that zoospores occurred in desmids. Archer noted that naked bodies emerged through tubes which had grown out from *Docidium ehrenbergii* = *Pleurotaenium ehrenbergii* (Bréb) De Bary, at the junction of the two semi-cells; these bodies encysted and again emerged as ovate or pyriform, ciliated swimmers.

West & West (1906) identify a fungus found in *Pleurotaenium ehrenbergii* with that of Archer. They, however, mention that the thallus is divided into

two parts by a septum. It is possible that this septum may be an artefact, since, according to their figure, if a septum is present only the portion of the thallus in one semi-cell has dehisced, whereas that in the other semi-cell having no exit tube, should still possess its contents, which are not figured. Both these records are incomplete, and until the structure of the resting spore is known their actual identities remain uncertain.

If Wests' fungus is divided into two parts it would then resemble the form described by Tokunaga (1934) in *Surirella* sp. and *Navicula* sp., where the thallus is septate at indefinite intervals into a number of cells, each component cell functioning at maturity as a sporangium or oogonium. As pointed out by Sparrow (1943, p. 537), such septate fungi cannot be included in the Ectrogellaceae as originally defined by Scherffel (1925) and may represent the type of a new genus. Karling (1942), however, has revised Scherffel's original diagnosis of *Aphanomycopsis bacillariacearum* to include the septate form described by Tokunaga which is considered by the author to represent at least the type of a new species.

### III. *ANCYLISTES* spp.

The genus *Ancylistes*, although known for a long time from the Continent (Pfitzer, 1872), has only recently been discovered in America (Berdan, 1938), and until now has not been recorded from Great Britain. On the two occasions I have found *Ancylistes* it was rare and only a few stages in the life history were observed. In view of this, I refer to them as *Ancylistes* spp. although it is possible that one may be new to science.

Since Berdan (1938) removed *Ancylistes* to the Entomophthorales, due to the discovery of aerial-borne conidia, no further support of her work has appeared in the literature. In the one specimen I found parasitizing *Closterium* sp. from the Clay Pond, Wray Castle, in September 1946, I too observed such conidia (Fig. 4).

Up to the present, species of *Ancylistes* have been recorded only in *Closterium*, whereas the second fungus, which occurred on a muddy path leading to Three Dubs Tarn, Claife Heights, near Sawrey, Lancashire, parasitized *Tetmemorus granulatus* Bréb. Although *T. brebissonii* was also present, it was not attacked. The specificity of these organisms was also noted by Berdan, for *Ancylistes closteri* and *A. pfeifferi* occurred together in the same pool but attacked different species of *Closterium*.

The fungus in *Tetmemorus* differs from other species since all the external hyphae grow to the centre of the desmid and then push out through the broken junction of the two semi-cells (Fig. 5). No conidia or resting spores were observed.

The intramatrical mycelium is unbranched, often extending to the length of the host cell, 98–115  $\mu$  long by 10–17  $\mu$  broad with rounded ends and containing regularly arranged refractive granules. More than one infection may occur in a single host cell (Fig. 6e, g, h). At maturity, the thallus is cut up into three to twelve segments, 9–25  $\mu$  long by 10–17  $\mu$  broad. Each segment produces one external hypha which grows rapidly, forming septa posteriorly and contains highly refractive granules collected together in certain regions (Fig. 6h, i). Branched external hyphae are commonly seen. One thallus was found which did not become cut up into



segments, and produced a single broad external hypha (Fig. 6*h* (x)). Infection of new hosts is by the swollen end of an external hypha in contact with the wall. The appressorium is very weakly developed and in many it could not be found. Soon after the host cell is entered the infection tube disappears. Early stages in development are shown in Fig. 6*a-c*. The

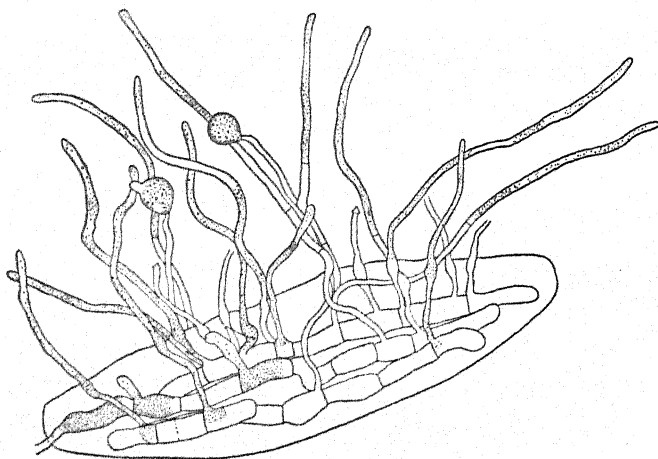


Fig. 4. *Ancylistes* sp. with external hyphae and conidia in *Closterium* sp. from the Clay Pond, Wray Castle.  $\times 256$ .

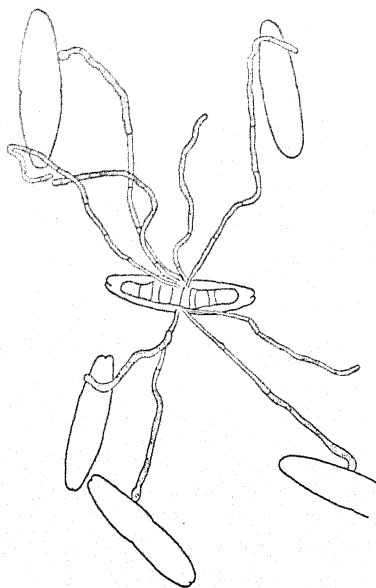


Fig. 5. *Ancylistes* sp. in *Tetmemorus granulatus*. Infection of new host cells by external hyphae.  $\times 150$ .

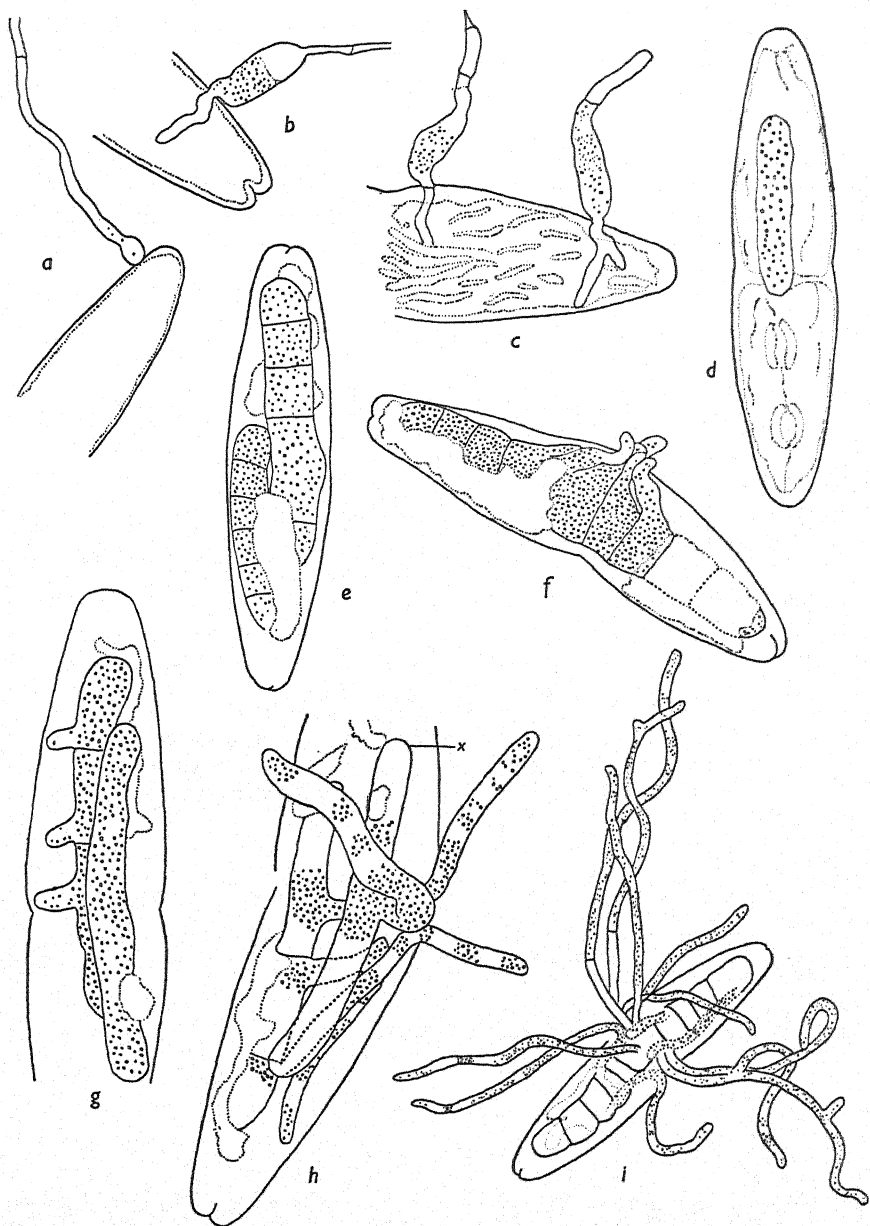


Fig. 6. *Ancylistes* sp. a-c, early stages in infection by external hyphae. d, young mycelium. e, two mycelia which are divided into segments. f, g, mature mycelia producing external hyphae. h, non-septate thallus at x producing a single broad external hypha. i, empty mycelium with growing external hyphae. a-h,  $\times 500$ ; i,  $\times 300$ .

ovoid ball-like structure formed by the rapid entrance of the protoplasm of the swollen external infection cell into the desmid was not observed. It is only when further stages in the life history of this fungus are known that its affinities can be discussed.

## SUMMARY

*Aphanomycopsis bacillariacearum* has been found in *Pinnularia* sp. from Blelham Bog, near Wray Castle, and a new species *Aphanomycopsis desmidiella* is described parasitizing the desmid *Netrium digitus*. *Ancylistes* is recorded for the first time from Great Britain and the presence of a conidial stage is confirmed. *Tetmemorus granulatus* Bréb. is a new algal host for this parasite and the emergence of external hyphae at the junction of the two semi-cells of the desmid has not formerly been described.

My grateful thanks are due to Miss E. M. Wakefield of Kew, for the Latin diagnosis, and to Prof. C. T. Ingold for reading the manuscript.

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# NOTES ON TWO STRAINS OF *FUSARIUM* *OXYSPORUM* FROM THE OIL PALM IN THE BELGIAN CONGO

By T. GOGOI

*Department of Cryptogamic Botany, University of Manchester*

(With Plate XVII and 6 Text-figures)

## INTRODUCTION

The two strains of *Fusarium oxysporum* considered here were isolated from the Oil Palm (*Elaeis guineensis*) by Prof. C. W. Wardlaw and identified by Dr W. L. Gordon of the Dominion Laboratory of Plant Pathology, Winnipeg (Wardlaw, 1946*a, b*). One of these strains was associated with a wilt disease characterized by necrotic discoloration of the vascular strands of the trunk and roots in palms of four to twenty years of age. The other strain was isolated from a characteristic leaf disease known as 'patch yellow', certain genetical types of palm being apparently highly susceptible. As noted above, both strains have been identified as forms of *Fusarium oxysporum*.

Four isolations from necrotic vascular strands and three from diseased leaves were available for study. Preliminary investigations indicated that the several isolations of each group were identical, but the two groups described here as *Fusarium* W (from wilt) and *Fusarium* P (from 'patch yellow' leaf disease), though closely comparable in some respects, were different in others. On some media, for example, the two strains look very much alike. Among the writer's aims, therefore, was to find a synthetic medium (or media) which (i) would serve to distinguish the two strains, and (ii) would be suitable for the manifestation of the principal morphological characters of the strains.

In this paper an account is given of preliminary mycological investigations of the two strains. The investigation of diseases of the oil palm, an important source of vegetable fats and oils, is still at an elementary stage. Although the pathogenicity of the two strains to be considered here has yet to be established, it is a matter of much interest that *F. oxysporum*, the causal organism of many important tropical diseases, has been observed associated with two very different and apparently unrelated pathological conditions in the oil palm.

## METHODS

The experiments were based on single conidial cultures. No sectoring was observed in either of the strains.

As one of the main objects was to find a medium or media which would serve to distinguish the two strains, a large number of different media and variants thereof were used, including malt agar, Czapek-Dox agar, modifications of Brown's synthetic agar, Sabouraud's agar, and potato agar.

Conidial suspensions in sterile physiological saline solution were used for the inoculation of Petri dish cultures. The densities of the conidia in the

suspensions were adjusted after haemocytometer counts had been made; the plates were inoculated with a platinum loop.

Observations were made on the appearance of cultures, the development of colour in the medium, rate of linear growth, size and shape of conidia, occurrence of chlamydospores, etc. In general, the experiments were carried out in triplicate at 24° C.

### *Observations*

*General.* On the several different media used the rate of linear growth was practically identical in the two strains. On most of the media the two strains were not identical in appearance, but the differences between them were often slight. Some media, however, brought out quite definite differences. Characters in which marked contrasts were observed in different media included (a) pigmentation, and (b) amount of aerial mycelium. These were recorded in cultures two weeks old and again after four weeks, the two sets of observations being closely comparable. The colours (Ridgway, 1912) shown by the strains on different media are set out in Table 1.

*Characterization of strains on a standard medium.* In the light of much preliminary experimental work I eventually selected the following modified recipe of Brown's synthetic medium for this purpose: glucose, 2 g.; potato starch, 25 g.; sodium nitrate, 2 g. (asparagin was not available); tri-potassium phosphate, 1.25 g.; magnesium sulphate, 0.75 g.; agar, 15 g.; water, 1000 ml. Media with 5, 7.5 and 10 % potato starch were also found to be satisfactory for normal growth. The data obtained with the selected standard medium after two weeks are set out in Table 2.

The mycelial growth of strain W was thinner in modified Brown's synthetic medium with lower concentrations of potato starch, although it was more dense than that of strain P. The pigmentation produced by the strains when different concentrations of potato starch were used varied: it became deeper as the concentration of starch was increased (Pl. XVIII, figs. 1-4). In comparable cultures of the two strains the pigmentation developed earlier in strain P than in strain W, but later the pigmentation in strain W was deeper than in strain P.

In strain P both the total sporulation and the proportion of microconidia to macroconidia were increased as the concentration of potato starch in the medium was decreased, i.e. septation increased with increase in starch concentration. Microconidia were predominant in strain W over the whole range of starch concentrations tested. Chlamydospores were abundant in strain W in the lower concentrations of potato starch, and also appeared in higher concentrations as the cultures became older. Chlamydospores were not observed in strain P in the higher starch concentrations. In general, the conidia of strain P show much more curvature than those of strain W (Text-figs. 1 and 2). This is at once apparent when the two strains are cultured on potato blocks.

From these observations it appears that strain P responds more readily to changes in the medium than does strain W.

*Growth on modified Brown's synthetic medium with different concentrations of glucose.* When glucose was used in place of potato starch a very light pink

colour was produced by both strains at the several concentrations after two weeks. At higher concentrations of glucose the colour gradually changed

Table 1. *Colours (Ridgway) produced on different media*

Medium	Strain W	Strain P
Malt agar	Pale 'pinkish buff'	'Deep hellebore red'
Sabouraud's agar	No characteristic coloration	'Rocellin purple'
Potato agar	Pale 'drab grey'	'Smoke grey'
Rice (whole)	Light pink becoming 'shell pink'	'Claret red' becoming 'Pompeian red'
Brown's synthetic medium (modified with rice starch)	Light pink; later becoming 'indigo blue' at higher rice concentrations	Light pink; later becoming 'indigo blue' at higher rice concentrations
Czapek-Dox agar	No characteristic coloration	No characteristic coloration
Brown's synthetic medium (modified: no potato starch)	No characteristic coloration	No characteristic coloration

Mycelial growth in strain W was stronger than that in strain P in all the media used except the synthetic media which gave very thin growth.

Table 2. *Characters of strains on the standard medium*

	Strain W	Strain P
Mycelium	Luxuriant aerial mycelium	No aerial mycelium: pionnotal development
Pigmentation of medium (after two weeks)	'Indigo blue' colour in patches near centre extending towards periphery*	Ring of 'indigo blue' colour extending from about 2 cm. from centre towards periphery (see Pl. XVIII)
Pigmentation of medium	Entire culture 'dull violet black' (after four weeks)	'Flesh pink' colour associated with whole culture; 'indigo blue' colour more marked
Sporulation (based on 500 conidia)†	Microconidia approx. 60 %	Microconidia approx. 30 %
Septation of macroconidia	Macroconidia from 1- to 7-septate; 1- to 5-septate conidia predominant; range of length of 3- to 7-septate conidia, 15 to 54 $\mu$	Macroconidia from 1- to 9-septate; 1- to 5-septate conidia predominant; range of length of 3- to 9-septate conidia, 15 to 39 $\mu$
Curvature of macroconidia	Less curved	Much curved, becoming sickle-shaped
Attachment of conidia	Microconidia and macroconidia directly abstricted; no secondary branches (Text-fig. 5)	Microconidia abstricted from tips of short secondary branches in rapid succession, accumulated and formed into spherical heads; macroconidia not in heads (Text-fig. 6)
Chlamydo-spores	Abundant	Very few

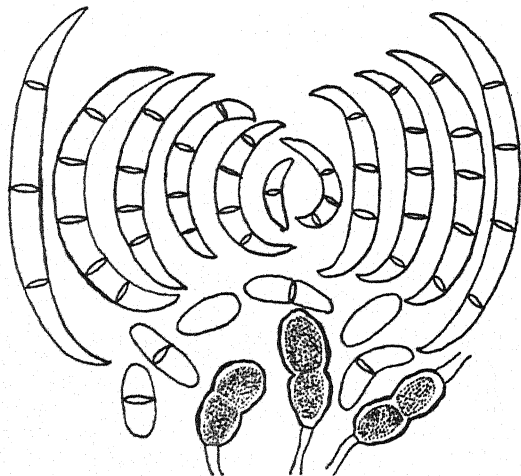
\* N.B. When strain W was cultured on this standard medium several months later, the blue colour did not develop in the standard medium containing 2.5 % starch, but developed slowly in the higher starch concentrations. This is now known to be due to the replacement of the wild type by mutant forms.

† Conidial samples were taken 1 cm. from the centre of cultures two weeks old.

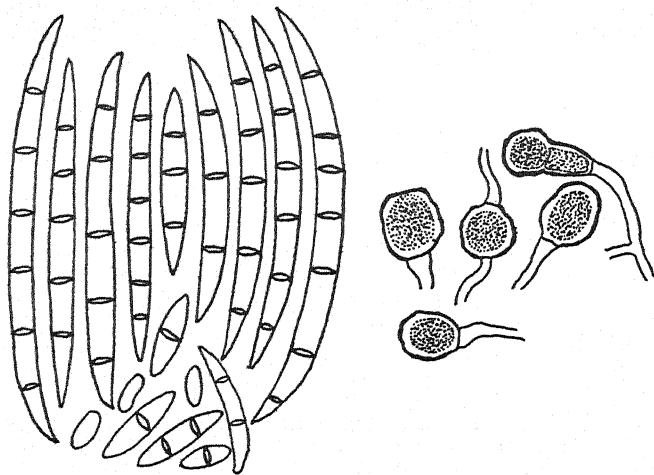
to reddish purple and finally 'claret red'. The 'indigo blue' colour observed in media containing potato starch was not observed. It can therefore be concluded that the 'indigo blue' colour in the medium is associated with the breakdown of the starch into sugar.

*Growth on modified Brown's synthetic medium with different concentrations of rice starch.* Similar results were obtained when the two strains were grown on modified Brown's synthetic medium with different concentrations of rice

starch. A light pink colour was produced by both of the strains over the whole range of concentrations. 'Indigo blue' colour was observed in the media with higher starch concentrations after two weeks. The amount of sporulation and the septation of conidia in the two strains on these rice



Text-fig. 1. A group of conidia and chlamydospores of strain P. ( $\times 1000$ .)



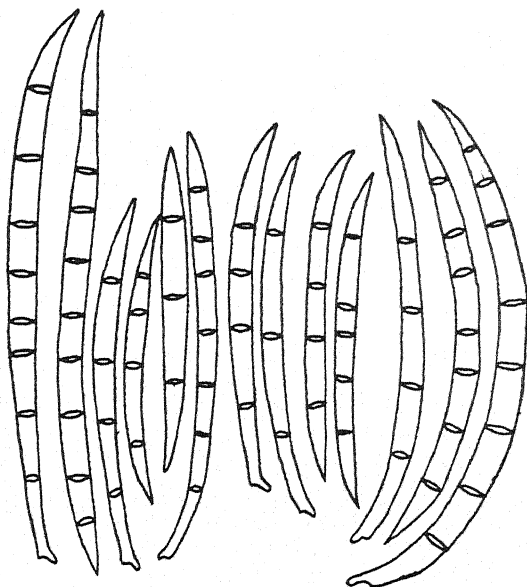
Text-fig. 2. A group of conidia and chlamydospores of strain W. ( $\times 1000$ .)

media were closely comparable with those observed in media containing potato starch. But in the matter of curvature, whereas at low concentrations of rice starch (0.05 %) strain P showed greater curvature than strain W, this was reversed at high concentrations (15 %).

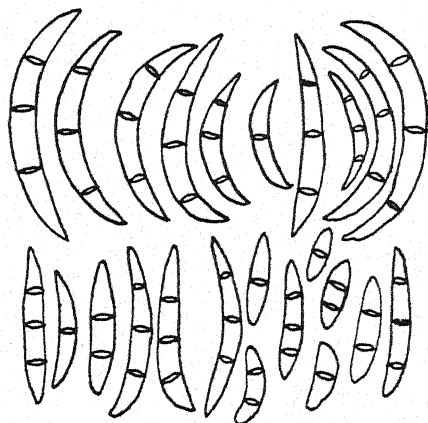
*Growth on soil.* As strain W was associated with a vascular wilt disease, and therefore presumably soil-borne, the investigation was extended by growing both strains on a sterilized loam soil.



In each instance the fungus spread slowly over the surface of the soil and down into it. Microscopic preparations of the aerial mycelium after two weeks showed that macroconidia were predominant in strain W. They



Text-fig. 3. A group of macroconidia of strain W showing high septation. ( $\times 1000$ .)



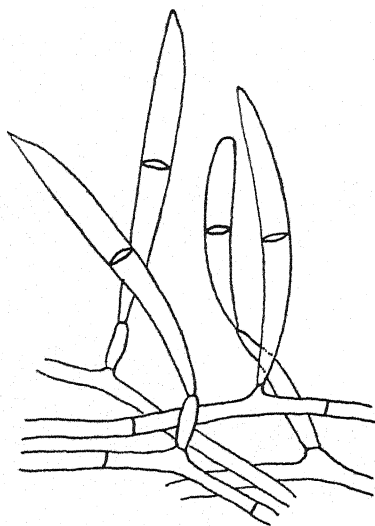
Text-fig. 4. A group of macroconidia of strain P. ( $\times 1000$ .)

were very long and from  $16.9 \times 2.8$  to  $70.3 \times 3.4 \mu$ ; 4- to 8-septate conidia were common (Text-fig. 3). Macroconidia and microconidia were present in equal proportions in strain P (Text-fig. 4). The macroconidia of strain P measured from  $8.4 \times 2.8$  to  $33.7 \times 3.4 \mu$ . They were of different sizes and shapes. The macroconidia were from 1- to 6-septate, 1- to 3-septate conidia

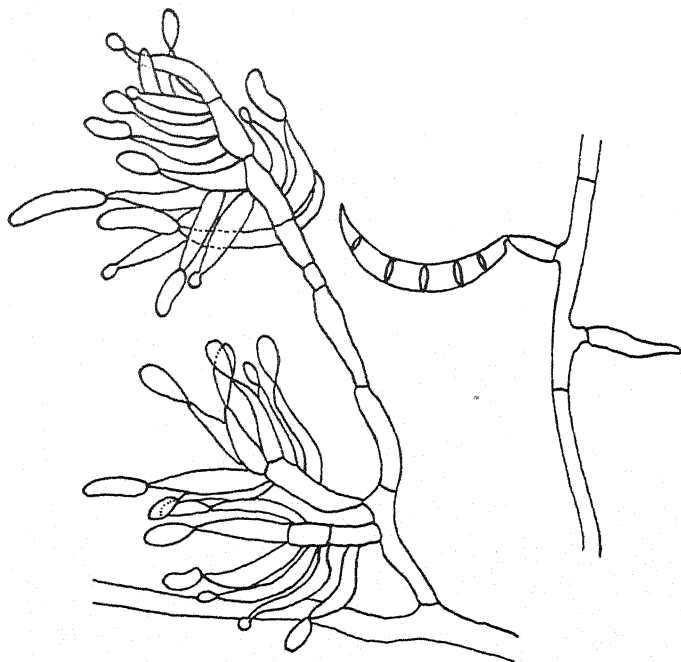
being common. Some of the highly septate conidia were typically sickle-shaped and with well-defined apices and pedicels. On starch-containing media, as described in the previous section, the conidia of strain P were larger than those of strain W. On this soil medium the reverse was found. In both strains only surface mycelium yielded conidia.

The strains were grown on an aqueous extract of the loam stiffened with agar, the pH being adjusted to 5.6. Probably this medium was too dilute, for only a very thin non-sporulating mycelium was obtained.

*Effect of light and darkness on the growth of the strains.* A striking difference was noted when the two strains growing under comparable conditions were exposed to light and darkness on a synthetic medium containing glucose, potato starch and the usual mineral ingredients. Strain P consistently showed zonations in those cultures which had been exposed to the light and an absence of zonation in those in the dark; but strain W showed no zonation under either condition.



Text-fig. 5. Conidia and conidiophore of strain W. ( $\times 1000$ .)



Text-fig. 6. Conidia and conidiophore of strain P. ( $\times 1000$ .)

## DISCUSSION

The observations show that the two fungi, which have been identified as strains of *Fusarium oxysporum*, though closely comparable in some respects, are not identical. Thus, on the same media, differences were observed in the pigmentation produced in the medium, in zonation when exposed to light, in the amount and appearance of the mycelium, and in sporulation. Furthermore, under certain cultural conditions, the two strains showed appreciable differences in the taxonomically more important characters of size, shape, septation and manner of abstriction of their conidia. These characters carry much weight, especially in the system of Wollenweber and Reinking (1935). On some media, however, the two strains were very much alike, for example, on modified Brown's synthetic medium and on Czapek-Dox's medium. It has been found convenient to select a particular medium as a standard medium: this serves to show the contrasted characters of the two strains. Actually, by suitably modifying media, the two strains can be made to approximate very closely in their morphological and physiological characters.

A difference of marked interest was noted when the two strains, grown under comparable conditions, were exposed to light and darkness. Strain P showed zonation in those cultures which had been exposed to light. This observation confirms those of Snyder and Hansen (1941*b*), who recognized the fact that light is the only one factor in the environment which may cause zonation. But strain W showed no zonation whatsoever.

The results of this investigation are of interest in relation to previous work on the classification of the genus *Fusarium*. The two strains can be separated by several morphological characters of the kind used and considered valid by Wollenweber and Reinking (1935). But, according to Snyder and Hansen (1940, 1941*a*, 1945), these morphological characters are unstable and unreliable as taxonomic criteria. These authors also consider that the species of *Fusarium* which cause wilt diseases are merely biologic forms of one and the same species, i.e. *F. oxysporum*. In short, the several forms or strains are to be recognized by their specific pathogenicities. As a practical issue, it should, however, be noted that much time will probably elapse before the relevant experiments on pathogenicity can be carried out with the oil palm. In the meantime it may be anticipated that, as this disease becomes more fully investigated in different regions, further isolations of *Fusarium* will be made. If so, it will clearly be desirable to have some standard with which these new isolations can be compared. Hence, the importance of a knowledge of the morphological and physiological characters of the strains so far obtained.

Studies of the pathogenicity of the two strains, strain P on leaves, and strain W as affecting the vascular system, will be awaited with interest. Reciprocal inoculations will clearly be both of academic interest and practical importance. Indeed, it is probably rather unusual to find two strains of an organism like *F. oxysporum* associated with such very different manifestations of disease as in the present instance.

## SUMMARY

1. An account is given of two strains of *Fusarium oxysporum* which were isolated from leaves and from diseased vascular strands of the oil palm (*Elaeis guineensis*) in the Belgian Congo.
2. An account is given of the growth, colour reactions and sporulation of the two strains on a selected synthetic culture medium. Other cultural tests by which the strains may be recognized and compared are also indicated.
3. When the two strains were grown under comparable conditions differences were observed in pigmentation, in zonation when exposed to light, in the amount and appearance of mycelium, and in sporulation; but media could be selected in which the two strains appeared identical.
4. The relation of these results to taxonomic aspects and future work on such *Fusaria* as may be found associated with these diseases of the oil palm is discussed.

The writer wishes to express her thanks to Prof. C. W. Wardlaw for suggesting this work, and for his helpful criticism during its progress, and to Mr E. Ashby for the preparation of photographs.

*Postscript.* After this manuscript was sent to press, it was found that the wild type of strain W is subject to mutation, though not of the sectorial type. It is now known that the large macroconidia shown in text-figure 3 are of mutant type. These matters will be described in a later paper.

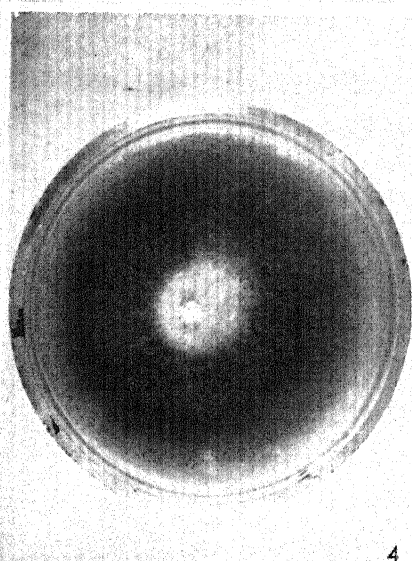
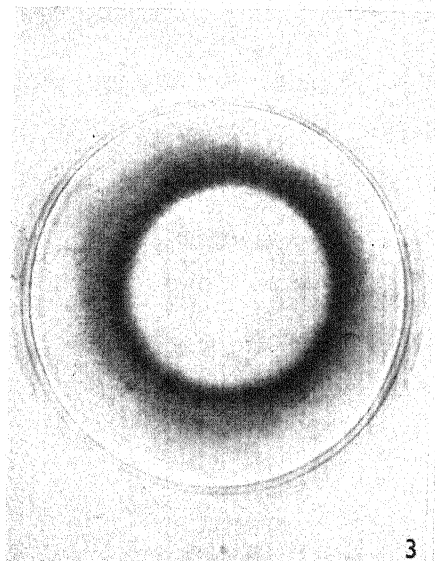
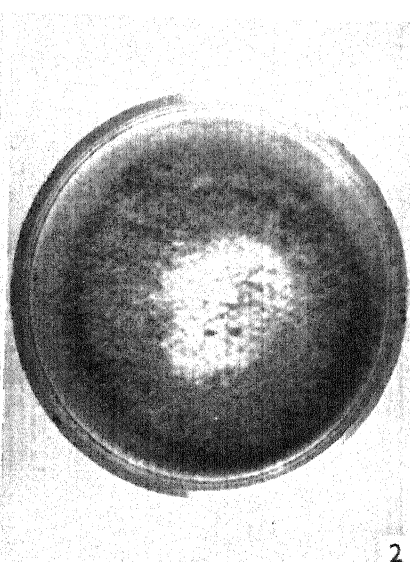
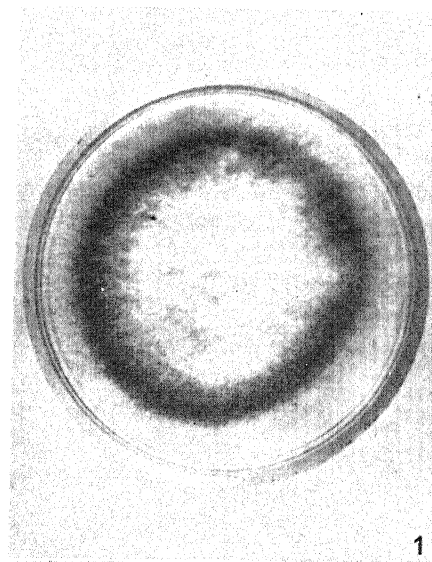
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## EXPLANATION OF PLATE XVII

- Figs. 1, 2. Cultures of strain W in modified Brown's synthetic medium with 2.5 and 10 % potato starch respectively, showing luxuriant mycelial growth and development of 'indigo blue' colour.  
 Figs. 3, 4. Cultures of strain P on same media showing scanty mycelial growth and development of 'indigo blue' colour.

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## THE ECOLOGY OF FUNGI WHICH CAUSE ECONOMIC PLANT DISEASES\*

By A. S. BOUGHEY, *University College, Exeter*

(With 2 Text-figures)

Ecological investigations on fungi have not developed on anything approaching the scale of those on higher plants. They have been confined chiefly to studies on terricolous Agarics, where it has been shown (Wilkins, Ellis & Harley, 1937; Grainger, 1945) that distribution is determined by the nature of the substrate, this depending on the character of the subsoil, the type of higher plant dominants and ground cover, and climatic factors such as temperature and moisture.

At the same time there has been a vast increase in the number of plant pathological papers. It has been estimated (Foister, 1946) that some 10 % of this literature is concerned with ecological investigations on particular organisms. The plant pathologist is now in fact compelled to study not only the etiology of a disease but also the epidemiology of its causal organism. The data on the autecology of individual fungi thus accumulating will ultimately prove invaluable when a comprehensive ecological review of the whole group is attempted. In the meantime it is interesting to examine whether synecological data may be extracted from such work on the regional distribution of fungi as has already appeared.

### DIFFICULTIES OF SYNECOLOGICAL FUNGAL STUDIES

There are several immediate difficulties inherent in any synecological study of fungi. First, as fungi are not free-living organisms, their distribution is restricted to the geographical range of their particular host or substratum. As a result of this dependence on living or dead organic matter, the relationship between individuals and between species is less direct than that found in a higher plant community. In a closed community of higher plants both individuals and species compete, primarily for light, water, and nutrient salts. Such a community of directly competing individuals and species is less common among fungi, occurring chiefly in the soil, where the phenomenon of competition is called antagonism, and among wood-invading and aquatic fungi. A third difficulty arises because a considerable part of the life history of a fungus may be spent within another living or dead organism, where the physical and chemical features of the environment are not easily measured.

### SYNECOLOGICAL STUDIES ON FUNGAL PATHOGENS

These difficulties may to some extent be avoided, as was first pointed out by Butler (1925), by considering only those fungi which cause diseases of *cultivated* plants. Wild species of higher plants are usually restricted to

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a narrow range of habitats, those in which they can maintain themselves against competition from other wild plants. Their fungal parasites are therefore of necessity restricted to the same narrow range. Parasites of cultivated plants, on the other hand, suffer no such narrow limitation, for cultivated plants are often grown under a very wide range of climates and soils. It is therefore possible to examine the behaviour of these fungi under widely contrasted environments.

Environment will react strongly on a parasitic fungus only at certain phases of its existence. For the most part the fungus will be growing within its host, in a constant environment modified only by relatively small changes resulting from interaction between the host and its habitat. To be a successful parasite of a cultivated plant, a fungus must not only be able to establish itself and live within this constant host environment, but must survive the periods when it is exposed outside its host. There are usually three such periods. The first occurs during long-distance dispersal, for to be a successful parasite the fungus must have some effective method of spreading itself widely, to become ubiquitous. The second is during out-of-season survival, for there are few agricultural areas of the world not subjected to periods of excessive heat or cold, or severe drought or flood. The last and most important period of all will be during short-distance dissemination, when the fungus is spreading rapidly and locally, enabling the disease to reach epiphytotic proportions. This dissemination stage is as vital in the development of a fungal pathogen as the seedling stage is in the higher plant, and it is therefore on this stage that environment will have its greatest effect.

#### SHORT-DISTANCE DISSEMINATION TYPES

The fungi which cause diseases of cultivated plants can be conveniently classified into seven major groups according to their method of short-distance dissemination: Powdery Mildews, Downy Mildews, Rusts, Smuts, Fungi Imperfecti, Pyrenomycetes, and Hymenomycetes. These groups are taxonomic units, not all of the same status, which have been defined in the first place on the characters of their sexual spore stages. Nevertheless, each group shows a remarkable uniformity in its method of short-distance dissemination.

Powdery mildews are disseminated by means of air-borne conidia. These are short-lived, rarely surviving more than forty-eight hours, and in most instances so far investigated appear to be capable of germinating in air of any atmospheric humidity, but only with difficulty in water (Berwith, 1936; Yarwood, 1936; Hashioka, 1937; Longree, 1939; Stoll, 1941; Brodie & Neufeld, 1942; Cherewick, 1944).

Downy mildews are disseminated by means of sporangia, in a few instances reduced to conidia. The sporangia appear to require the presence of free water for some three to five hours before they can germinate, liberate their zoospores, and establish a fresh infection (Müller, 1930; Richards, 1939; Magie, 1942; Yarwood, 1943).

Rusts are disseminated, as far as those causing diseases of cultivated plants are concerned, principally by means of uredospores. These are relatively long-lived spores, remaining viable usually for some two or three

months, and requiring, in such as have been examined, the presence of free water or a saturated atmosphere before they will germinate (Beauverie, 1924; Goldsworthy & Smith, 1931; Hemmi & Abe, 1933; Yarwood, 1939; Straib, 1940).

Smuts are usually disseminated by means of chlamydospores, which are long-lived, but their mode of infection is not so uniform as in the other six groups. It is apparently assumed that these chlamydospores require free water to bring about germination, for there seems to be no record of investigations on their germination in the dry state.

Fungi Imperfecti have been shown to fall into two groups, according to whether their conidia can be scattered dry by air currents or must first be wetted (Mason, 1937), but conidia of both types apparently require free water, or a relative humidity of over 90 %, before they will germinate (Barrus, 1921; Stevens, 1932; Dennis & O'Brien, 1933; Vestal, 1933; Wellman, 1934; Inoue, 1937; Katsura, 1937; Burke, 1938; Guba, 1938). It is possible that wetting with water is always an essential preliminary to germination, as was shown for *Diplocarpon rosae* by Lyle (1938).

From the plant pathological point of view Pyrenomycetes form an unsatisfactory group, for their perfect stages are often saprophytic or rare, and can play no major part in the short-distance dissemination of the parasite. For ecological studies it is possibly better to include the Pyrenomycetes under Fungi Imperfecti, but for present purposes they have been treated as a separate group.

Hymenomycetes are disseminated by air-borne basidiospores. Like the chlamydospores of smuts these basidiospores are apparently always assumed to require the presence of free water or a nutrient solution before they will germinate.

Short-distance dissemination will, however, involve not only the question of spore germination but also that of spore production. This appears to be influenced by water and air humidity in much the same way as spore germination. Thus, production of conidia by powdery mildews is depressed in the presence of free water or very high atmospheric humidities (Hashioka, 1937), while that of sporangia in downy mildews (Weston, 1923; Yarwood, 1937, 1943; Ogilvie, 1944; Beaumont, 1947) and conidia in Fungi Imperfecti (Petri, 1931) is favoured.

It appears also that successful infection is dependent on much the same moisture conditions as lead to optimum spore germination, for it takes place most readily under dry conditions with powdery mildews (Yarwood, 1936; Hashioka, 1937), under wet conditions or in high atmospheric humidities with downy mildews (Schultz, 1937; Ogilvie, 1944), rusts (Fromme, 1913; Lauritzin, 1919; Goldsworthy & Smith, 1931; Asuyama, 1939), and Fungi Imperfecti (Muncie, 1917; Lauritzin, 1919; Barrus, 1921; Dastur, 1921; Van der Muyzenberg, 1932; Stahel, 1937; Burke, 1938).

It is therefore not surprising to find that outbreaks of disease epidemics have been correlated with the occurrence of rainfall or dews in downy mildews (Salmon & Ware, 1931; Crosier & Reddick, 1935; Moore, 1937; Schaal & Edmundson, 1943), rusts (Johnston, Melchers & Miller, 1938; Belin, 1938; Matheny, 1939; Melchers & Johnston, 1939; Craigie, 1945)

and Fungi Imperfecti (Barrus, 1921; Dastur, 1921; Elliott, 1922; Stevens, 1932; Wellman, 1934; Guba, 1939; Hendrickx, 1942). Moreover, as the presence or absence of free water or high humidities has this controlling influence over the short-distance dissemination phase of fungal pathogens of cultivated plants, some degree of correlation may be expected between rainfall and the geographical distribution of economic plant diseases.

#### DISTRIBUTION OF SUDAN PLANT DISEASES

The diseases of cultivated plants in the Anglo-Egyptian Sudan have been recorded on a regional basis (Boughey, 1946) to facilitate subsequent ecological analysis. It has been shown (Boughey, 1947) that the distribution of the pathogens (fungi, bacteria, and viruses) which cause these diseases is positively and significantly correlated with the amount of annual rainfall. The number of diseases in the wettest areas is approximately three times that found in the arid ones.

It is interesting to examine the distribution of the Sudan fungal pathogens in each of the seven groups previously described (Fig. 1a). Powdery mildews predominate in the arid areas, gradually decreasing with increasing rainfall to reach a very low percentage representation in the wettest areas. With Fungi Imperfecti the exact opposite is found, diseases caused by this group predominating in the wettest areas, then decreasing, until they are almost completely absent from the driest area. Downy mildews tend to increase with increasing rainfall, while rusts first decrease then increase. Other groups, including the miscellaneous fungi belonging to unclassified groups, show no consistent variation.

The system of disease recording just described has not been used in any other country. It is not therefore possible to compare the Sudan results, as regards fluctuations in the total number of pathogens, with those from other sources. The method of analysing the composition of the plant-disease flora, on the other hand, can be applied to any comprehensive plant-disease list, and direct comparison made.

#### DISTRIBUTION OF SCOTTISH PLANT DISEASES

A list of Scottish plant-disease records on a regional basis has been produced by Dennis and Foister (1942). As the regional basis for their list they used a previous subdivision of Scotland into twelve main drainage areas. Five of these areas, Orkney, Sutherland, Ross, Hebrides, and Argyll, have too few records for them to be included in any analysis. The other seven can be divided into three groups, a low rainfall east-coast group (Moray, Dee, Forth, and Tweed), a high rainfall west-coast group (Clyde and Solway), with Tay having an intermediate rainfall.

The percentage representation of each of the seven classes of fungal pathogens in each of these three rainfall groups is shown in Fig. 1b. Much the same trends can be observed as with the Sudan data in Fig. 1a with the addition that smuts and Pyrenomycetes also appear to decrease with increasing rainfall. The trends are not nearly so marked as with the Sudan data, but the rainfall differences are very much smaller.



## DISTRIBUTION DATA FROM A REPRESENTATIVE SERIES OF PLANT-DISEASE LISTS

Instead of considering data from individual countries such as the Sudan or Scotland, the plant-disease lists of scattered countries may be used, provided that these countries form fairly uniform areas and that their lists are reasonably complete. Lists from seven areas were selected as meeting with these requirements, and providing a sufficient contrast in the amount of annual rainfall. These lists were for Egypt (Reichert, 1921), Morocco (Maire & Werner, 1937), Mauritius (Shepherd, 1937), Tanganyika (Wallace, 1937), Britain (British Mycological Society, 1944), the Bombay province of India (Uppal, Patel & Kamat, 1934), and the Connecticut State of the U.S.A. (Clinton, 1934).

The relationship between the mean annual rainfall and the composition of the plant-disease flora of these areas is shown in Fig. 1c. Comparing it with the Scottish list illustrated in Fig. 1b, the same general trends can be discerned although, as must be expected with lists from such varied sources, there are a number of anomalous points.

## DISCUSSION

It has not been found possible to provide this present examination of plant-disease floras with a statistical basis. Nevertheless, the existence of similar trends, in data from three quite different sources, affords some considerable support for the existence of a real relationship between the annual rainfall of a given area and the proportion of fungal pathogens in each of the seven major groups which have been described. It appears that this proportion can be calculated from the annual rainfall alone, without reference to any other factor such as air temperature. Thus a temperate area, such as the Solway-Clyde area of Scotland, may have much the same group representation as a tropical island like Mauritius, which has something approaching the same mean annual rainfall in its agricultural areas. This similarity is illustrated in Fig. 2a. That is not to say, of course, that *individual* fungal species will be unaffected by temperature, for temperature is often quite clearly the main factor determining *specific* distribution.

The simplest explanation for this relationship between group distribution and annual rainfall may perhaps in some instances be the correct one. If powdery mildew conidia are not produced and do not germinate abundantly in the presence of free water, powdery mildews would be less numerous in countries with a high rainfall. Similarly, if water is necessary for the production and germination of the sporangia of downy mildews and the conidia of Fungi Imperfecti, it is not surprising to find these two groups better represented in the higher rainfall areas. With this simple explanation, however, rusts and smuts should increase with rainfall too, while actually they decrease. With rusts, perhaps some host factor comes into operation, or possibly the heavy dews which are so characteristic a feature of arid agricultural areas may replace rainfall. Such an explanation would satisfactorily account for the distribution of rusts in the Sudan, where rusts are best represented in both the wettest and driest districts. The relationship between smuts and their environment is bound to be a more complex one because of their varied methods of establishing infection. Whatever

the true explanation for the relationship, it lies beyond the scope of the present paper, which is concerned merely with the demonstration of the relationship.

Given this rainfall-distribution relation, it is possible to construct a typical profile for an arid area, and for a very wet area, areas of intermediate rainfall having correspondingly intermediate profiles (Fig. 2*b*). The arid profile, obtained by combining the disease lists from the arid provinces of the Sudan and Egypt, is characterized by a great predominance of powdery mildews, a fair representation of rusts, smuts, and

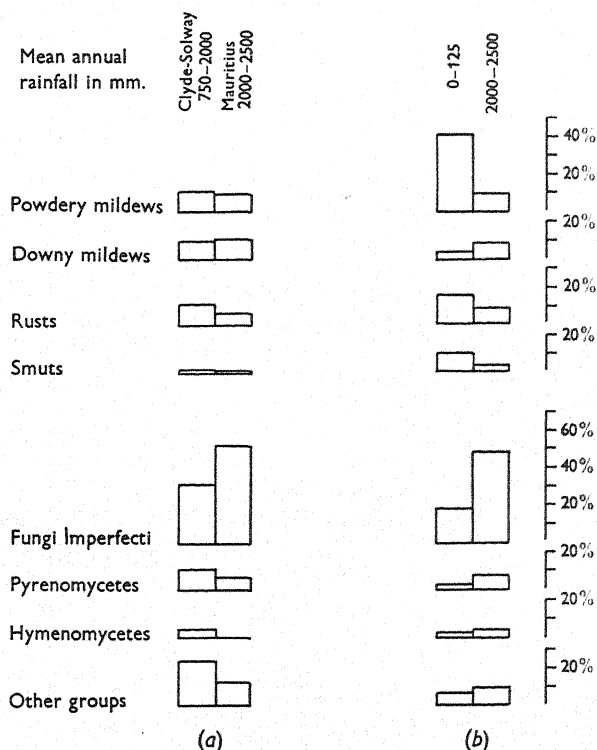


Fig. 2. Proportional representation of fungal groups in (a) two areas of approx. similar rainfall, (b) hypothetical very dry and very wet areas.

Fungi Imperfecti, with low numbers of downy mildews, Pyrenomycetes, Hymenomycetes, and miscellaneous fungi. The wet profile, obtained by uniting Mauritius and the Solway-Clyde area, has a very high percentage of Fungi Imperfecti, increased representation of downy mildews, Pyrenomycetes, and miscellaneous fungi, with lower numbers of powdery mildews, rusts, and smuts, and still low figures for Hymenomycetes.

It should be possible to find some better measure of the amount and duration of the wetting with free water to which crops will be subjected than annual rainfall. The total number of days in the year when rain falls or dew is deposited, for instance, might provide a better assessment of this

wetting. Also, in this paper no attention has been paid either to annual variations in the distribution of the fungi which cause diseases of cultivated plants, or to fluctuations in the *incidence* of such parasites. While both are directly related to, and will have an important effect on, the regional occurrence of plant diseases, it is believed that their proper consideration will not radically change the general conclusions as to fungal ecology which have been presented.

There are several implications arising from this ecological study in regard to agricultural and horticultural practices. First, the greatest proportion of seed-borne diseases of economic plants belong to the Fungi Imperfecti. As this group is most strongly represented in wet areas, which appear to have in any event larger numbers of diseases than dry ones, seed production, from a plant pathological point of view, should be concentrated in the driest parts of a country. The United States administration has already made use of this suggestion, but other countries appear slow to follow suit. It is true that there is a concentration of seed production in the eastern counties of England, but this appears to have arisen for other reasons. Secondly, when rainfall is supplemented by irrigation, the method of applying the water is of some plant pathological importance. Where overhead irrigation, instead of flooding or subsoil irrigation, is used, a wet type of climate will be simulated in an otherwise dry area, and troublesome diseases not normally present in the area may occur. Where overhead irrigation must be used, a fungicide could perhaps be added to the water. The third point is rather similar. Plant-house watering should be arranged as far as possible to avoid wetting foliage.

In conclusion, while it seems that lists of economic plant diseases can be analysed to provide data on the synecology of fungi, their value in this respect would be greatly increased if variable areas could be subdivided into districts showing some uniformity, and if diseases which are absent, as well as those which are present, could be recorded.

#### SUMMARY

There have been comparatively few synecological studies on fungi, possibly because difficulties arise from the dependence of fungi on living or dead organic matter, the frequent absence of competition, and the problem of assessing habitat factors. By considering only those fungi parasitic on cultivated plants, these difficulties can be partially avoided.

An analysis of Sudan plant disease records shows that the number of pathogens increases with increasing rainfall, powdery mildews predominating in arid, Fungi Imperfecti in wet areas. Similar trends can be detected in Scottish records, and in a set of disease lists from seven representative countries.

The *proportion* of powdery mildews, downy mildews, rusts, smuts, Fungi Imperfecti, Pyrenomycetes, Hymenomycetes, and the remaining fungi in an economic plant disease flora can apparently be estimated from annual rainfall alone.

Implications regarding agricultural and horticultural practices are suggested.



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## PROCEEDINGS

## MYCOLOGICAL EDUCATION

Meeting held in the Department of Biology, Chelsea Polytechnic, London, at 11.0 a.m., 31 January 1948. The President, Prof. A. E. Muskett, in the Chair.

G. C. AINSWORTH. The Society and mycological education.

Although the British Mycological Society does not profess to be an educational organization, education is implicit in many of its activities. During the past fifty years the Annual Autumn Forays have played a major role in training successive generations for the study of our larger fungi and in maintaining the so-called 'tradition of species'. Since 1918, the Plant Pathology Committee has done much towards educating plant pathologists to treat the names of plant pathogenic fungi with greater respect and understanding. But every member, whether professional or amateur, who has made any progress with a study of mycology would probably have to admit a greater or less debt to the Society—and there are very few British mycologists whose names have not appeared in the Society's membership lists.

About five years ago the Society felt concern at the state of systematic mycology in this country and the Council appointed a Committee to investigate the position and make recommendations. In June 1944 the committee's report was printed (but not published), and in the following month copies were distributed to government departments, universities, research stations, and other bodies interested in mycology.

The work of preparing that report raised the question of the training of much needed systematists. At the same time the war had emphasized the importance of mycology in diverse fields and focused attention on the absence of educational facilities for those wishing to take up such mycological work. The Council therefore set up a second committee to make recommendations regarding the teaching of mycology. Much valuable information was derived from a questionnaire sent out to all university departments, agricultural colleges, and other teaching institutions known to be interested or potentially interested in some aspect of mycological teaching and the committee's report, like the earlier report, was printed but not published and widely circulated in June 1946\*.

There have been various developments during the last eighteen months but the position cannot yet be viewed with equanimity and this discussion provides an opportunity for an exchange of views on mycological education between mycologists of widely differing interests.

W. G. KEYWORTH. Mycology for the plant pathologist.

Experience is the greatest factor in producing a good Plant Pathologist, and it is inadvisable to lay too much stress on a detailed preliminary training. Nevertheless, it is obviously necessary for a pathologist to have as good a training as possible, and I propose to give a few of my own views on how this training should be devised.

At present most Plant Pathologists take a pure Science degree in Botany or an applied degree in Agriculture or Horticulture. In the pure Science degree, mycology is taught in much the same way as other aspects of Botany, with emphasis on the classification of the families which show features of taxonomic interest. In general the treatment of mycology in most University Degree courses is adequate for a preliminary training, but there must of necessity be many gaps. After taking his degree, the future pathologist often undertakes two years' research for a higher degree. This research is generally confined to a very restricted branch of the subject and, because of the demands it makes on the worker's time, there is little or no opportunity for broadening his general knowledge of mycology. The student may then become either an assistant in the Advisory Service or a research worker. If he becomes an adviser he obtains much practical

\* These two reports are printed in the *Transactions*, xxxii, 100-112.

knowledge in the course of his duties, and after a time may become reasonably well versed in his subject: if he becomes a research worker he usually continues to specialize and has little opportunity of learning the broader aspects of mycology.

Many of the attributes of a good adviser cannot be learnt in any course, but must be inborn or be acquired by constant and sometimes somewhat painful contact with growers. On the mycological side, however, the main requirement seems to be a considerable skill in diagnosis, for which a wide knowledge of symptomatology is required. He has to be adept at identifying fungi, often from somewhat unsatisfactory material, and with the aid of books of reference which are not always very helpful. Having identified the cause, he must be able to indicate the best means of control, if any, and have a considerable practical knowledge of the difficulties which may be inherent therein.

The research worker has to be rather differently equipped. While generally specializing in some narrow branch of mycology, he must be capable of applying the results of other workers to his own problem. He must be skilled in the technique of studying a fungus *in vitro* and also in its host, be able to conduct careful inoculation experiments, and be conversant with the effects of environment on both pathogen and host.

Is the present system of training adequate for either of these workers? The degree training in mycology would not seem to help either very much, although necessary as a basis for further work. The subsequent concentration on a piece of research for a higher degree may be of value as a training in observation and experimental method, but it leaves the adviser with an incomplete general background and may make the research worker concentrate too much on special methods which he may not require thereafter.

How can these difficulties be overcome? I think both types of worker should continue their study of general mycology for some time after taking a degree. During such study, emphasis could be placed on pathogens, but not to the exclusion of all others, as these may often be encountered by pathologists in many branches of their work. All too often the Fungi Imperfecti are ignored because they have no life history or other taxonomic aspects of interest, but the group contains a very large number of important pathogens as well as many saprophytes normally encountered by pathologists. I suggest that in any scheme of training for pathologists thorough attention should be paid to this group, and to the methods by which it is classified. Included in the general mycological training there should be an adequate course on classification and taxonomy, with some insight into the problems of systematics. The student should also have ample opportunity of finding his way about the various keys which exist for identifying fungi, if only to learn the pitfalls that may beset him later. An opportunity should also be given for students to study the growth and physiology of a representative selection of pathogenic fungi, both *in vitro* and on their hosts, as well as the simple and more complex methods of isolating fungi.

After such preliminary training the courses for the two types of pathologist might diverge. The prospective adviser could, with great benefit, spend some time at an Advisory Centre to learn the practical problems, and to take an intensive course on the recognition in the field of diseases and their control. The research worker could concentrate on a study of research methods and technique in relation to a range of pathogens, supplemented by a small research programme.

Courses such as I have indicated are already given at one or two Universities in England, but they are generally undertaken during the degree courses and are therefore somewhat hurried. Post-graduate courses in pathology somewhat on these lines appear to be quite successful in America. I suggest, therefore, that consideration be given to the institution of a post-graduate training course for pathologists which would meet the requirements of the several types of worker, and which would incorporate the studies that experienced pathologists regard as of most value. Such a course might profitably lead to a recognized higher degree or a diploma in the subject, which would become accepted as a sound preliminary training.

#### F. D. ARMITAGE. Mycology for the industrial microbiologist.

There appears to be no course of instruction in applied mycology or any diploma in industrial mycology available to the staffs of industrial laboratories in this country. Such facilities are available in bacteriology, and the instruction includes descriptions of some of the common micro-fungi, chiefly to draw the attention of students to differences in morphology and nutritional requirements.

The microbiologist employed primarily to control the manufacture of a commodity produced by a fermentation process is usually a qualified biochemist who is prepared to make a special study of the physiology of the organisms of most interest to him by post-graduate study and research. On the other hand, anyone in an industrial laboratory who wishes to acquire a working knowledge of systematic mycology, in order to become familiar with the many kinds of micro-fungi which may spoil industrial products, has to rely almost entirely for mycological education on the information to be gained from reading the existing literature, and this is not an easy task, particularly without the guidance of one already conversant with mycology and mycological nomenclature. Indeed, to read the literature without such expert guidance tends to give the newcomer to mycology an impression of constant conflict between mycologists, who seem unable to agree for long on the family relationships of the specimens they so fondly foster.

This Society could therefore render great service to industry if it used its wide knowledge and influence to bring into being a school of mycology that would include, as part of its curriculum, tuition suitable for mycologists in industrial laboratories.

Apart from a general knowledge of the physiology of moulds and of their industrial uses and applications, the worker in the technical laboratory requires proper instruction in laboratory technique, with particular attention to the preparation and maintenance of culture collections. Methods of sampling technical products for mycological investigation should be standardized in principle, so that some measure of the degree of infection of a product can be accurately assessed. Instruction should also be given in experimental methods for comparing the value of industrial fungicides, for the inhibitors and methods used in phytopathology are often not applicable to industrial products. The correct use of inhibitors became a very important part of packaging research during the war years, particularly for materials going abroad and the export drive is now making the matter still more urgent. Some insight should be given into the morphology and cytology of the organisms to be studied as well as sufficient initial training to enable the industrial mycologist to place a fungus in its proper genus. It would also be helpful if the Society could organize a series of lectures later to be published and designed specifically for those beginning the study of micro-fungi.

### I. MARTIN-SCOTT. Mycology for the medical practitioner.

Although fungi are less important as pathogens of man than of plants, their role as disease-producing agents was first recognized in animals and man. In 1835 (more than ten years before Berkeley's classic paper on Potato Blight) Bassi demonstrated that one organism could enter another and cause disease, when he showed experimentally that a fungus caused muscardine disease of silkworms. Four years later Schoenlein described the fungus of favus in man; and shortly afterwards, *Microsporium audouinii*, a cause of ringworm of the scalp in children, was described by Gruby in France. These results stimulated research and speculation on the part fungi played in other human diseases, until later in the century the work of Pasteur, Koch and Lister emphasized the predominant role of bacteria in their causation. Subsequently, medical mycology received little attention until the eighteen nineties when the work and enthusiasm of Sabouraud in Paris led to similar investigations both in this country and elsewhere. These researches, chiefly on ringworm, were undertaken by medical men who had apparently few contacts with the non-medical mycologists of their day. In England, Colcott Fox, Blaxall and Adamson, among others, made notable contributions to medical mycology. Colcott Fox described and named *Trichophyton sulphureum*, which is still recognized as a 'good' species, and he and Blaxall also gave the first description of the cat ringworm fungus.

After 1895-1905, the heyday of British medical mycology, the subject fell into a decline and most doctors to-day are very ignorant of this interesting subject: an unhappy state that has come about because of the medical student's overcrowded curriculum and the poor facilities for post-graduate study. Medical mycology is not yet a subject in the undergraduate's already overloaded curriculum. During his six years' medical study ringworm infections are briefly outlined in the systematic lectures in dermatology: this amounts to about one hour's instruction. Thus the newly qualified doctor knows nothing of the microscopic mysteries of the mentagrophytes, or the perplexing problem of pleomorphism. He appreciates in a vague sort of way that ringworm

may affect the scalps of children, and that the macerated grey soggy skin between the toes of his beach companion is due to a fungus.

If he takes up Public Health work he will receive a short course of lectures on medical mycology, and those medical men who practise dermatology find a knowledge of the dermatophytes imperative. Facilities for post-graduate study in mycology in this country are conspicuous by their paucity. The remedies are plainly obvious: the future medical student should be able to receive systematic instruction in mycology just as he receives instruction in bacteriology at the present time, and for the post-graduate, centres of mycology should be founded in the main cities where teaching could be undertaken and facilities offered to those interested in research.

These suggestions imply close collaboration between medical men and mycologists. 'Medical fungi' are much like other fungi, and it is rarely possible for a doctor to become familiar with more than the clinical aspects of the subject. The outstanding advances which have been made in the United States in the mycology and epidemiology of mycotic infections by the close collaboration of medical men and mycologists is a lesson we have yet to learn in this country.

### J. M. B. KING. Mycology for the amateur.

In the field of phanerogamic botany amateurs can be divided into two classes—the one content to recognize and name the commoner flowers, and the other going much more deeply into species, varieties and forms and having some knowledge of plant physiology. These divisions seem hardly to exist in the mycological world. The extreme difficulty of identifying fungi, together with the absence of suitable books and practical help, soon discourages the faint-hearted and only the more determined enthusiast survives, the practical result being a small but select group that takes its mycology seriously. Such a group should receive every help and encouragement, but it would be wrong to ignore the needs of the weaker groups.

Briefly, the needs of the amateur are literature and personal contact. Even in normal times the shortage of suitable books has been a serious handicap to the student of fungi, and to-day recourse must usually be had to second-hand bookshops where prolonged search may occasionally unearth a copy of Massee, Cooke or Berkeley. Even so, such works are long out of date, often inaccurate and seldom include microscopic data such as spore size, cystidial characteristics and so on. Our classic stand-by for the larger fungi—Rea's *British Basidiomycetes*—is unobtainable and unlikely to be reprinted. One of the few books available to-day is Ramsbottom's *Handbook of the Larger British Fungi*. Unfortunately this contains little microscopic data and no coloured illustrations, both of which are essential for successful diagnosis. Certain foreign books can be obtained, notably Lange's *Flora Agaricina Danica*, but they are usually expensive and difficult to obtain. Under literature three important requirements are glossaries, colour charts and keys. Glossaries should form a part of nearly every scientific work, and certainly every work designed for students, for if the reader knows less of the subject than the author, then it may be assumed that he also knows less of the terms. Authors seem often to overlook the fact that though a student may be familiar with general mycological terms, he may be ignorant of the special terms used. Let us too, have liberal translations of Latin and Greek terms. We cannot all be classical scholars and an immense amount of time can be wasted in searching through dictionaries for meanings. There is every reason for generic and specific names to be in Latin, though in an English work English equivalents should be included, but I can see no reason for Latin and Greek in other respects. Diagnostic keys are favourite places to sprinkle with Latin tags. For 'carnosae', 'coloratae', 'albatae', 'megasporae', 'mesosporae', 'stenosporae', would it not be just as easy to say 'fleshy', 'coloured', 'whitish', 'large spores', 'medium spores' or 'narrow spores'? Even Lange's masterpiece, a work obviously designed for English-speaking peoples, is irritatingly profuse in such terms.

Closely following the need for new books with good glossaries comes the need for good colour charts. It is often extremely difficult to describe the colour of a fungus and the difficulty is added to by the use of such inexactitudes as 'alutaceous', 'isabelline' and 'ochraceous'. For my part I never know whether an author means red or yellow ochre when he speaks of 'ochraceous' and as for the tans and tawnys and livids, they cover a vast range of tones. Would it not be possible for the British Mycological Society to



publish in the *Transactions* a standard colour chart? What a boon it would be if it could be decided once and for all what tint was intended by 'ochraceous'; it would matter little whether this was red, yellow or even blue or green as long as we all understood the same thing.

From colour charts we come naturally to keys. Here there is immense scope for improvement. The simple dichotomous systems used so successfully with flowering plants are less easily applied to fungi and we still await the perfect system, but meanwhile the task of identifying fungi is not made easier by the constant shifting of species from one genus to another, or the creation of new genera in the ceaseless attempt to find a natural classification. As an example of this I may mention *Armillaria mucida*. This species has now been placed by Lange in *Collybia* and becomes *Collybia mucida*. In making this change one of the fundamentals of every known key is shattered—the presence or absence of a ring. How can simple and usable keys be evolved when exceptions to their very foundations have to be made? I see only one solution to the problem—two systems of classification. One as straightforward and simple as possible, designed for the express purpose of identifying fungi, the other—the natural system—for pure science. I see little objection to such a duality, for once a specimen had been diagnosed by the simple system it could readily be referred to its position in the natural system if necessary. In devising any revised system of classification a very necessary first step is a clean sweep of the numerous ghost species now cluttering up practically every text-book. Many fungi given specific rank could probably be reduced to varieties or forms and by thus thinning out or condensing the present long lists of species, which most mycologists have never seen and probably never will see, the task of diagnosis would be made much simpler.

Of even greater benefit to the amateur would be the allocation to his special needs of a few pages in each issue of the *Transactions*; short but authoritative articles on the elements of mycology—e.g. cytology, taxonomy, classification, generic notes—would be extremely welcome and go some distance to meet the shortage of up-to-date literature.

The other important requirement of the amateur is personal contact. In the study of any subject books in themselves are insufficient; there comes a time when practical guidance and help is necessary. Those of us who study the larger fungi well remember the days when returning from the woods with a basket of specimens we attempted the hopeless task of reducing the confusion to order. It soon became apparent that no advance could be made without the finger of experience to point the way. Forays are one form of personal contact: they are an essential and vital part of the Society's activities, providing an opportunity not only to meet experienced mycologists but to benefit from that experience on the spot. I admire the careful way in which these forays are conducted and particularly the manner in which the specimens are laid out and labelled at the end of the day: and I know of no other Society that does this.

Forays apart there exists only one other opportunity for personal contact and that is in the meetings of the Society. These meetings are delightful in character, and provide a blend of science and sociability. As an amateur, however, may I make one criticism: almost invariably these meetings deal with micro-fungi and related matter of an advanced character, and I often feel how much I would like to hear the same speakers lecture on the larger fungi or on taxonomy, classification, cytology or micro-fungi from a humble aspect. The amateur admittedly forms but a small part of the Society, but the experts may not be experts in every branch of mycology and an agaricologist would doubtless enjoy a simple talk on micro-fungi and vice versa. Personal contact for the amateur takes the place of the lecturer and demonstrator of the professional student and I know this society will never express towards its humbler members the sentiment I heard recently in another place 'Oh, we can't be bothered with the rabble'.

#### C. G. C. CHESTERS. Mycology in the Universities.

*Orthodoxy.* Mycology has been, and still is, regarded as a part of the curriculum of a botany course in a University. In most British Universities a student who desires to specialize in mycology arrives at his goal by way of an honours course in botany followed by graduate research and with the ultimate qualification of a M.Sc. or of a Ph.D. in botany. From first to last the word mycology does not enter into the documented qualifications with which he is credited, except in so far as testimonials or references from the department of the University concerned may indicate the line of specialization which

he has followed, and except in so far as the particular department has an external reputation for that type of specialization.

This is the accepted course and it will continue to be accepted in a number of Universities. It can be justified and it has many points of value. We believe in Britain that specialization at the undergraduate level is not desirable; that we should not train for professional careers as such; that we should provide a liberal education. We have inherited the view that fungi are members of the vegetable kingdom and they are usually closely associated with the algae in the phylum Thallophyta. This acceptance of a point of view has resulted in potential mycologists being more or less well versed in the field of botany, perhaps in the whole field of botany, and it has resulted in many advantages for the mycological student, as a mycologist or as a would-be plant pathologist. He has been required to know plants and this should have been of immense value in the study of organisms which frequently live on plant substrates. The knowledge he has acquired in plant taxonomy, anatomy and physiology can all be brought to bear on his future selected topic of study. This line of study has a tradition behind it and much can be made of its underlying principles.

As an initial point of discussion I believe that the fungi should be removed from the Thallophyta and should be treated quite independently of the algae both in taxonomic position and time of appearance in the syllabus. The following outline appears to me to be a desirable method of treating the fungi within the syllabus of a botany honours school. During the intermediate course (if such a course continues to be given in the Universities) the student should encounter the fungi principally from the standpoint of life-cycle studies in relation to their importance to man with sufficient emphasis on their structure, function, reproduction, genetics and classification to impress on him their biological peculiarities and their importance both to the life of the green plant and to the animal kingdom. In position, such a treatment should follow a résumé of the phyla of the plant kingdom. Passing to the honours school the student is destined to spend three years in the study of plants, and I am presuming that during the first two of these years he will cover the whole of the subject-matter in a general way, and that during the last year he will return to selected topics for discussion in greater detail. In such a course the precise location of his studies of fungi becomes important. I believe it should follow his consideration of the physiology and the anatomy of the green plant and should therefore come within the second year of that course. The subject-matter should be based essentially on the study of the taxonomy of the fungi, and should deal with the separation of the larger units of the class as illustrated by named species, studied as far as possible in the fresh condition on their characteristic substrates. Every effort should be made to give a good, reasoned account of the class, bringing into the scheme relevant facts on the physiology and the genetics of species. Students should be encouraged to collect and to study the fungi in the field and should be led to realize that fungi are to be found in specific localities growing under specific conditions. When the final year is reached the curriculum of each student may be varied according to certain selected interests. The student is now able to devote each day to botanical studies and at this stage I should introduce a short course of bacteriology covering the principles and the technique of this subject. This is followed by studies of the physiology of fungi, of the detailed taxonomy of specific families as exercises in the application of criteria for the recognition of species, of the genetics of fungi, of the economic importance of fungi to mankind and of the principles of isolation, cultivation and preservation of fungi. The student might be required to attend lectures elsewhere in the department on plant pathology and to spend a week in field work dealing with the collection and identification of fungi. A short investigation of a specific topic or the discussion of a particular field of literature might well be introduced.

Graduate studies can be built on such a foundation and I should plead here the importance of graduate studies in formal mycology as a further course for certain professional careers rather than the immediate undertaking of research projects in mycology. I am certain that there is a necessary place for graduate diploma courses in mycology in the training of the student who has passed through the curriculum of an honours school of botany, and such advanced formal studies are stimulating both for student and teacher. The suggestion that a Master's degree in mycology might well be a first research degree has been made in this Society's report on the Teaching of Mycology and I believe this to be an excellent step in the training of a professional mycologist.

*Heterodoxy.* Why not break with tradition and come right out into the open with the

declaration that mycology is a science of equal standing with bacteriology and, for that matter, with botany, and provide a course for an honours school in mycology? The background of the course, after intermediate or its equivalent had been passed, would be a one year subsidiary course in chemistry (or botany), along with two session courses in botany (or chemistry) and in mycology, followed by a final honours course in mycology. The outline of the initial mycological course would consist of a good grounding in bacteriology and in the physiology of fungi, followed by detailed consideration of the taxonomy of the groups of fungi. The final honours year would be devoted to intensive study of fungi both as individual organisms and as agents causing disease and as organisms capable of being induced to perform various organic syntheses. During this year organized field studies would play a large part in the course. It is not an impossible dream and, if we are to play our part in the world to-day, I submit that it must become a reality at some centre in Britain.

*Field work.* It may not always be possible to secure constant access to a reasonably private location near a University to permit continued and progressive study of the fungal flora by successive years of students, but there can be no doubt that such a system of field work is highly desirable. Similarly, an annual departmental foray gives the essential insight into field studies without which a mycological education is incomplete. As a complementary study to the fungi in the field the development of a teaching herbarium with well-documented collections of selected species within particular genera is an essential equipment of a mycological department. Such a herbarium should constantly extend and furnish a progressively expanding court of reference for the training in systematic mycology.

*Conclusions.* Whether a student be trained in the orthodox or the heterodox system outlined above there is one basic condition which must be fully satisfied. The study of mycology is rooted in a knowledge of the living fungus in its natural habitat, and by every available means—collecting excursions and forays of this Society—the student of mycology must be initiated to and raised on a study of the fungus in its natural association with the plant and animal life of the world.

#### L. M. J. KRAMER. Mycology in relation to schools.

Clearly the work of schools is predominantly that of education. Nevertheless, practically all secondary grammar schools and the newly set up modern schools make some provision for general science, while some, fortunately placed in point of situation, staff or facilities, emphasize biology or nature study and field work. Therefore some attention to mycology is possible, but obviously it can only be by passing reference or by elementary study of a few common moulds or higher basidiomycetes.

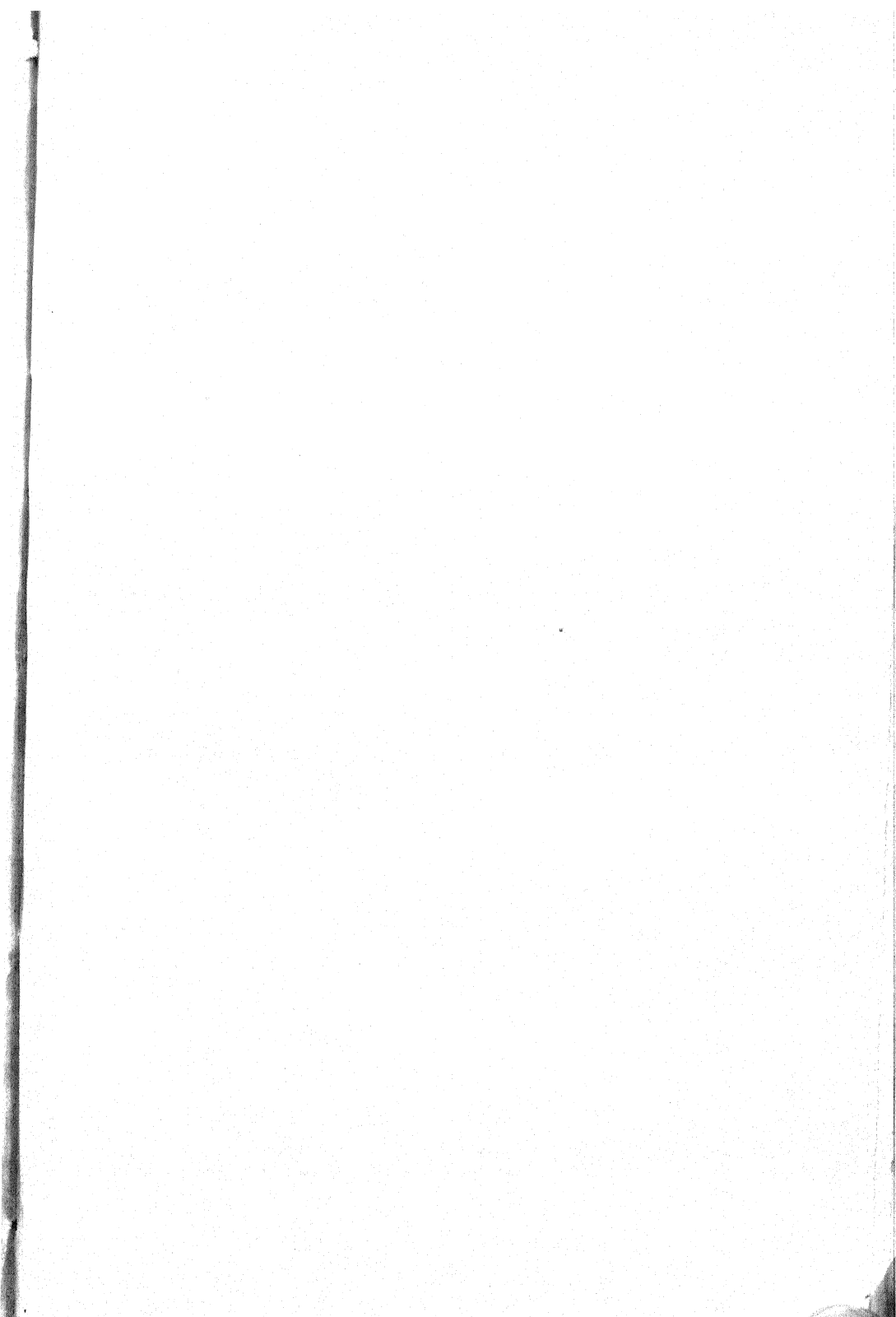
Beyond the standard of the School Certificate Examination much more time may be devoted to biology by candidates for Higher School Certificate or University Entrance Scholarship Examinations in Grammar Schools. Such pupils are the basic source of professional biologists, and their minds might suitably be directed to the implications of mycology and the possible future openings for those able to undertake the long training.

From the foregoing very brief statement of the present position certain suggestions arise. The advance of the research front cannot normally be followed adequately by school teachers, so that a method of informing them of recent trends, and of the need for change of emphasis of syllabuses, is called for. A medium for this exists, namely the *School Science Review*, the official organ of the Science Masters' Association. Articles at intervals on mycological matters would meet the need.

Advice on openings for mycologists and on suitable University and other centres for training could also be communicated to the *School Science Review*. The possible employment of non-graduates in a technical capacity in mycological laboratories should be borne in mind in preparing such communications.

For schools situated in large cities, where field work is extremely difficult, simple mycological work in laboratories is quite possible, and can serve to illustrate a number of basic principles of scientific experimentation. Notes on suitable work might perhaps be printed in the Society's *Transactions*.

The meeting concluded with a review by C. T. Ingold of the papers and subsequent discussion, in which he emphasized the important place that the Society holds in relation to mycological education and to mycological work generally, and in which he made suggestions for future progress in the different aspects of the subject.





PROFESSOR C. G. C. CHESTERS.  
President, 1947.

PRESIDENTIAL ADDRESS  
CONCERNING FUNGI INHABITING SOIL

By CHARLES G. C. CHESTERS

I well remember the impression which a conversation overheard in a certain botanical laboratory made upon my mind at the time when I finally decided to foray in search of mycological knowledge. Two friends were discussing certain mould cultures isolated from dilute suspensions of soil. One of these friends, who later became my much respected mentor in the field study of fungi, asserted forcibly, and with an emphasis of which he was a most effective master, that soil fungi as soil fungi did not exist and, further, that any attempt to identify a fungus in a test-tube was doomed to failure. It was against all the accepted tenets of mycological faith. Since, at that time, I was isolating fungi from small particles of soil plated out on agar these assertions shook me to the core. These isolations constituted an interesting pastime which appeared to be not devoid of possibilities. To have a senior colleague declaim in such a categorical manner made me pause and consider. In later conversations he expounded his views—that fungi were brought into the soil from above ground, that many so-called soil fungi were only temporarily resident in the soil, and that any fungi growing in that medium were in reality inhabiting particles of organic matter. Still, the urge to look below the surface stayed with me and at odd times drove me back to similar inspections.

The forays of this Society introduced me to methods of studying fungi inhabiting the surface of the earth, the bark and wood of fallen trees, and the waters of lakes and streams. At odd intervals, soil-inhabiting fungi were mentioned and, once in particular, a member of the Society quoted an American colleague as having stated that it was little use continuing to isolate moulds from the soil unless one knew that something was happening in that soil and unless one were isolating from that particular spot. It occurred to me then, and has constantly recurred to me since, that these words aptly describe precisely what a field mycologist does every time he collects a species or a group of species from a particular location. Experience has taught him to recognize some set of conditions in operation now, or the resultant of some past activities, which are associated with the appearance of the species. He is usually dealing with substrata of quite large size—pieces of material which can be picked up and handled. The fungus which they bear can be examined with a lens and, with its substratum, can be tucked away in basket, vasculum or envelope for further and more exact study. His collecting grounds include woods and meadows; orchards and arable fields—pleasant places, where he may wander and survey the prospect near or far of organic substrata on which the specimens of his species grow and flourish. Always his eyes are scanning the host or

the substratum for these conditions which will signify to him the likely presence of what he seeks. The habitat is the resultant of many factors, chemical and physical, and it is seldom static. This changeful nature underlies the succession of species which is an everyday experience to the field mycologist and which frequently indicates to him the presence or the future prospect of particular species.

Turn now to the soil and examine a vertical surface cut downwards through a pasture turf or an arable field in crop. Consider the upper 6 in. or so of this soil profile and there you have the major part of the compound habitat in which collection and study of soil-inhabiting fungi must occur. This is the micro-field in which our forays must be conducted. Superficial layers of superimposed plant detritus of varying age and of varying degrees of recognizable structure give place to more compact debris, which becomes more highly diluted by mineral particles and gradually loses structural features until visible recognition becomes impossible, and the only sign of organic materials is the darker colour of some soil particles. Penetrating downwards through these successions of layers are the roots of plants, some of which are alive and glistening while others are dead skeletons of their former selves. Occasional large fragments of recognizable stem, leaf or root material occur as wanderers far below the surface layer, having been brought down by animals into the soil. Moist air fills the cavities between the living roots, plant debris and mineral particles. Such is our field of exploration. Can you visualize the similarities and the contrasts between this location for a foray and the location for one of our annual excursions in search of fungi? In broad general principles our usual hunting grounds represent magnified, mirror images of the soil—the debris of fallen trunks and branches match in space, if not in substance, the leaf and stem fragments at the surface of a meadow soil and the leaf and twig debris at the surface of a wood; the tree trunks, alive or dead, mirror the root systems below the ground; these detached branches, perched in a tree, which, because of their inaccessibility, may be most prized and are generally most unproductive of fungi, correspond to those larger fragments of unspecified origin below the soil surface. It is not intrinsically so different though the materials are less durable, their size is smaller and the 'atmosphere' in which they exist is less variable. But these differences are sufficient to introduce noteworthy complications. In place of more or less sizeable fructifications, the investigator in this field is faced with microscopic assemblages of sporangia or conidia, of oospores embedded in plant debris and of minute perithecia with their contained ascospores—if he is lucky. More often he encounters sterile mycelium, frequently of a most obdurate and intractable kind which no amount of persuasion will persuade to fruit. Hardly collectable specimens—certainly not to be initially studied with a hand lens, removed and packeted, but none the less fungi, aping in their saprophytism and their parasitism; in their comings and their goings; in their fruiting and their dispersal, many of their larger relatives above ground. While their study should be approached in just the same way as that of fungi above ground, if maximum knowledge is to be accumulated of their effective life



in soil and of its implications to mankind, it is essential to realize that their identification is almost always a matter of difficulty, depending entirely on cultures established on sterile media.

How have we set out to investigate this field, what have we made of its problems, how have we studied its inhabitants? At the outset I want to make it perfectly clear that fungi are not the only tenants of this underworld just as they are not, thank goodness, the sole tenants of the world above ground. It is normally a well-balanced microcosm, but when unduly disturbed it has its storms and upheavals. Recent reviews by Burges (1939), Niethammer (1937) and Waksman (1944) have dealt with particular aspects of the distribution and the activities of soil-inhabiting fungi. I shall only deal here with limited aspects of the problem which appear to me to indicate future trends of development in this important section of mycology.

Sixty-two years ago Adametz (1886) initiated the study of soil-inhabiting fungi when he investigated the microflora developing in media sterilized in flasks and thereafter inoculated with soil, taken from the surface and from some depth in sandy and in loamy fields. From these flasks, inoculated in the open, he obtained eleven species of fungi. Koning, in partnership with Oudemans (1902), so improved the technique of isolation that his methods are worthy of more attention than they have been accorded. He inoculated plates of wort agar or wort gelatin with aqueous suspensions obtained by pulverizing fragments of vegetable matter extracted from soil in a given volume of water in a sterile crucible, and by diluting a portion of this suspension in sterile water. Some forty species were identified by Oudemans. To me this represents the first serious attempt to investigate the fungal content of soil, and it is the foundation upon which much recent work has been built. These investigations demonstrated the presence of moulds in soil of high organic content, particularly in relation to the organic matter, and indicated the appreciable variety of species which occurred in such conditions. Hagem (1908, 1910*a, b*) concentrated his attention on the Mucorales in Norwegian soils, and was the first person to plate out particles of soil on sterile media and to make transfers within a short period from the colonies arising from the inocula. In subsequent investigations the practice normally employed by bacteriologists for obtaining dilute suspensions was applied to the soils. Rivas (1910) was perhaps the first to introduce the method of collecting the soil sample by forcing a sterile tube into soil through a freshly exposed surface, and this method has been repeated by a great many investigators in soil mycology. C. N. Jensen (1912) employed a sterilized, tubular corer to obtain a cylindrical soil sample, due care being exercised to protect the sample from aerial contamination. He also reviewed previous records of fungi obtained from soil and presented a summary of the extent of our knowledge at that time. It is of significance that the study of fungi as organisms present in the soil had claimed few disciples as compared with the study of bacteria in the soil. In this science the method of progressive dilution and of plating soil suspensions had reached a very precise stage, and estimates of the numbers of bacterial units in known quantities of soil

had become a matter of routine. Conn (1917), who was a bacteriologist, asserted that it was an open question whether, in fact, fungi lived and grew in the soil and suggested that isolations from soil might only reflect the presence of dormant spores. Waksman (1916) had already shown that, using particles of soil as inocula on agar plates, fungi could be isolated within twenty-four hours from the colonies produced by incubation at about 30° C. He further compared the rate of growth from inocula of hyphae and from inocula of spores of the same species, and demonstrated that the former produced the larger colonies in an initial limited time period and therefore deduced that the earliest visible growth came from mycelium already present in the soil. In further studies, employing a technique for staining smears of soil, Conn (1922) obtained microscopic evidence for the presence of mycelium in soils of very diverse natures.

Meanwhile, quantitative data were accumulating regarding the so-called numbers of fungi in the soil (Waksman, 1922; Brierley, Jewson & Brierley, 1927). Most of these investigations depended upon essentially bacteriological methods which provided dilutions of known concentration relative to a given weight of soil. That these dilutions contained spores as well as fragments of mycelium was recognized (McLennan, 1928). The accumulation of this knowledge represented, and still represents, a phase in the study of soil-inhabiting fungi. In all biological processes, the investigator is faced with the evaluation of his observations in terms of units which permit comparison between sets of his data and those of other workers in the same field. Although a great deal of labour has been expended on the estimation of numbers of fungi inhabiting the soil, we are forced to recognize that these labours have been successful only in part. So long as we recognize the fact that, apart from the excellency or otherwise of the method of estimation, we are dealing in such studies with a limited and frequently highly specialized and highly fecund section of the fungal population, then we are justified in accepting the results. It must be clearly understood that, quite apart from the higher Basidiomycetes, such frequent inhabitants of the soil as species of *Pythium*, species of *Mortierella* and a galaxy of the darker Hyphomycetes are seldom, if ever, reflected in their true relationships in such studies. Brierley *et al.* (1927) made an exhaustive study of certain of the factors which influence the results of plate-counts of fungi obtained from soil dilutions and Brierley (1923, p. 123) had already pointed out certain factors which must be considered in evaluating such estimations. Not the least of these is the wide differences in the vegetative structure and in the spore-producing apparatus of fungal species which are both inevitably reflected in the degree of separation of the units in such dilution plates. Local and abundant sporulation influences the estimate of fungal numbers even in the largest and most random of samples which can be handled effectively under stringent aseptic technique. But despite these and other limitations it is quite clear that maximum population is attained close to the soil surface and that the population decreases with increase in depth. One of the potent factors in operation is the decrease in effective organic substrata on which the fungi can live, while other

factors include aeration, moisture, temperature and perhaps available soluble materials not related to the organic pabulum (H. L. Jensen, 1934). Lateral differences in fungal content are also experienced and are, in the main, exactly what has been experienced by any field mycologist as the natural state of affairs. It is very little use quoting precise examples of statements of the number of fungi per gram of soil because such figures, interesting and instructive as they may be, and I would not suggest otherwise, appear to be a compound of what is, what was and what may be. It is very difficult to visualize how this position may be remedied until a technique is to hand which permits easy and rapid visual examination and identification of these fungi which are growing in that same fraction of soil which is later diluted and plated. Circumstantial evidence has been obtained, however, and if sufficient data are accumulated it should lead to enlightened views on this subject.

Up to 1930, most of the investigations dealing with soil-inhabiting fungi were based on essentially bacteriological methods involving dilution of soil suspensions. The total sample was shaken up in water and that part which became free and remained suspended was introduced into agar and incubated. It was possible to give a quantitative statement of the number of fungal units isolated from a known weight of soil, always supposing each growing unit represented one original soil unit. Estimates of the numbers of fungi were obtained from soils differing in acidity and alkalinity; in organic matter of varying carbon content; in the level of nitrate, ammonium, phosphate; in moisture content; in the kind of cover vegetation and in many other respects. This kind of information indicated that viable fungal units can be extracted in no small measure from fertile soils containing organic materials, that the figures obtained varied with the richness of the soil in organic matter of different kinds, and that apparently the species of fungi did not differ much from soil to soil within a region, except in so far as species of *Penicillium* were commoner in temperate soils, species of *Aspergillus* in tropical soils, species of the Mucorales in woodland and cultivated soils and the general run of pathogenic species were only to be obtained in quantity where suitable hosts grew or where conditions favoured their continued existence. Valuable information was accumulated on diverse topics, but only a very indistinct picture of the fungus at work had been obtained; in fact, the working fungus was only really visible between the upper and the lower parts of a Petri dish or within the cylindrical wall of a culture tube. But, in 1928, Rossi devised one of those simple methods of biological experimentation which act as the trigger to set all sorts of reactions in motion. He pressed clean microscope slides against a freshly cut surface of soil and, after fixing this by heating, stained the preparation to display bacteria, fungi, and other members of the soil population in place relative to one another and to the soil particles. This was indeed an advance—almost like looking at the private life of soil organisms in a cinema 'still'. Cholodny (1930) modified this method by fixing the slide against the fresh soil surface with the loose soil removed to expose the surface, and by removing the slide for fixation and staining after a period of incubation. Conn (1932) applied this method to soil in vessels

so that the flora in different soils could be studied or the effect of different degrees of moisture and of different levels of fertilizer additions could be determined. He suggested that the colony formation of pure cultures in sterile soil could likewise be examined. The Cholodny technique was subsequently used in many soil studies (Demeter & Mossel, 1933; Garassini, 1939; H. L. Jensen, 1934; Timonin, 1940*b*; Verplanke, 1932; Ziemielka, 1935). It must be mentioned here, however, that reflexion on the scope and accuracy of this method raises doubt whether in fact it does picture the soil flora as it lives. True, no nutrient is added to the soil, but the glass surface allows moisture accumulation, permits local proliferation of bacteria and may even act as a surface for the transport and passage of fungal hyphae which is not normally present. It certainly disturbs the aeration of the soil. Nevertheless it has been modified and applied as a quantitative method by H. L. Jensen (1935), who obtained interesting indices of the relative richness of different soils in fungal mycelium. A further and a rather different method of estimation of the fungal mycelium in soil has been devised by Jones & Mollison (1948) in which small quantities of soil are pulverized in a small volume of water and the suspension so obtained is diluted with agar and from this dilution films of constant thickness are prepared, dried and stained.

None of these methods examines the living fungal population in situ. This has only been possible since the design and perfection of a system which allows vertical illumination of an object under examination, by high microscopic magnifications. Such vertical illumination was applied to soil studies by Kubiena (1932, 1935), and has resulted in the very interesting discovery of active growth of certain species which have not usually appeared in dilution plates. This method has definite promise, especially as it is possible to remove inocula from species seen growing in the soil-cavities using micro-instruments and to isolate from such inocula. I have adapted the Leitz Ultrapak for use in this method, but it must be clearly pointed out, however, that since the technique is laborious and since the results are not always of an equally high quality this method would appear to be better applied for the moment to limited and particular studies. It is decidedly not a routine method, but in the hands of a trained experimenter should yield interesting data not otherwise obtainable. The use of tubes with capillary orifices in the lower parts of the tubes and filled with sterile medium (either a nutrient agar or a mixture of soil and organic material) which are immersed in soil for an incubation period has already been described (Chesters, 1940, 1948). The species of fungi which are isolated from such tubes usually possess a widely spreading mycelium and grow actively in the soil. Although this method does not permit visual inspection of the soil for active mycelium there is little doubt that living hyphae must be present in the soil adjacent to the tube before the species can be isolated.

Taxonomic studies of species isolated from soil are to be found in many individual papers and not a few monographs. Species of the Mucorales have received much attention (Campbell, 1938; Dixon-Stewart, 1932; Hagem, 1908*a, b*, 1910; Jensen, C. N., 1912; Lendner, 1908; Niethammer,

1935; Pisppek, 1929), as have certain limited genera of the Fungi Imperfecti and a few Ascomycetes. Butler (1907) initiated a fresh approach specially profitable with species of *Pythium* and allied genera, and his methods of isolating such fungi from bait floating in water covering the experimental soil have been modified and applied by several investigators with most interesting results (Matthews, 1931; Middleton, 1943; Sparrow, 1943). From such studies, the range of species and the taxonomy of these species has been very fully considered. Gilman and Abbott (1927) and more recently Gilman (1945) have brought together most of the taxonomic literature on soil-inhabiting fungi in book form. It is still essential that more detailed studies should be undertaken, and I see little hope of stable and enduring work in this aspect of soil-inhabiting fungi until full documentation of isolated species by means of adequate descriptions, drawings, slides and dried culture specimens becomes a matter of routine. There is still an immense field to be investigated and many discoveries to be made.

Surveying the present state of our accumulated knowledge of the soil-inhabiting fungi, as discovered by the methods of investigation I have outlined, we are faced with the fact that most of this knowledge concerns species or genera in relation to gross habitat factors as reflected by their geographical range (Kursanoff & Stiklyar, 1938; Niethammer, 1935, 1937; Rayllo, 1928). Fungi have been isolated and described from, and their numbers have been estimated from, cultivated soils (Chand, 1937; Chaudhuri & Sachar, 1934; Ghatak & Roy, 1939; Jasevoli, 1924; H. L. Jensen, 1931 *a, b*, 1934; Singh, 1937 *a*; Ziling, 1932), from forest soils (Ellis, 1940; Paine, 1927; Pistor, 1930; Svinhufvud, 1937), from soils with high salinity (Bayliss-Elliott, 1930; Killian & Feher, 1935; Sabet, 1935) or with high organic matter content (Verona, 1934), from below particular kinds of cover vegetation (Deyl, 1938; Le Clerg, 1931), from cultivated as compared with natural soils (Bisby, James & Timonin, 1933; Dixon, 1928; Janke & Holzer, 1929; Ma, 1933 *a*; Samoutzevitch, 1927), from particular soils at differing seasons of the year (Dixon, 1928; Ma, 1933 *b*; Svinhufvud, 1937); from different depths in a particular soil, the depth being but seldom related to the soil horizons (Bisby, Timonin & James, 1935; Deyl, 1938; Jasevoli, 1924; Le Clerg & Smith, 1928; Paine, 1927; Swift, 1929; Todd, 1932). The relationships of fungi in the soil to organic matter (Thom & Morrow, 1937; Vandecaveye & Katznelson, 1940), to moisture and temperature, to soil fertility in relation to crop growth (Singh, 1937 *b*) have been discussed in several of the papers mentioned, but very little information can be obtained of exactly where in the vast expanse of the soil the fungi are actively growing—what precisely constitutes the individual habitat. The broad backcloth had been sufficiently painted in by 1916 for Waksman to be able to indicate the world-wide distribution of many species of fungi—some of them apparently not known from any other source. He could confidently portray a group of soil inhabitants remaining relatively constant in their occurrence in world-wide soils, but showing a replacement of species of *Penicillium* by species of *Aspergillus* in the warmer regions of the world. At times these local inhabitants were increased or modified by varying species of the common genera, and at all

times, alien species, not of this select group and evanescent in their stay within the soil, constituted the invader species. This idea of soil inhabitants and soil invaders is good just so long as you are content to work with gross isolation techniques, but one wonders if it will continue to hold when, as one hopes, soil will be subject to detailed collecting excursions. It is true that Reinking & Manns (1933) related these concepts to species of *Fusarium* in Central America and demonstrated for one genus that there are species so specialized in their parasitism that they cannot long survive as living units of the soil population apart from their host material, living or recently dead. Garrett (1938) very precisely defined these terms in relation to fungi attacking plant roots and has demonstrated the very real value of the concept in this field.

Let us examine the soil inhabitants for a few moments, but, before we do so, it is worth while remembering that even in 1947 the backcloth has a wide expanse unpainted. Basidiomycetes do not fully occupy even a corner of it, yet in the field they are obviously one of the very old inhabitants. Their isolation is a matter of getting the right technique—which so far has eluded us. In a survey of the lists of fungi isolated from soil it is still as true to-day as it was in 1916 that species of *Mucor*, *Penicillium*, *Trichoderma*, and *Aspergillus* predominate, and are closely followed by species of *Rhizopus*, *Zygorhynchus*, *Fusarium*, *Cephalosporium*, *Cladosporium* and *Verticillium*. When we attempt to generalize regarding the fungi isolated from particular vegetational units (e.g. forest, grassland, arable land) there is much contradiction and uncertainty as to the frequency of even the commoner species. It can serve little purpose to attempt a review of these here because the reasons for this variation are many and not the least of them is the method of isolation employed. It is worth pointing out that in many forest soils species of the Mucoraceae appear with great frequency and Hagem's (1910*a, b*) concept of a community of species of *Mucor* has been abundantly confirmed (Niethammer, 1935; Pistor, 1930). The dominant species is usually *M. rammanianus* in association with other species of this or related genera which vary from place to place. In general terms it may be said that species of *Mucor* and related genera are more frequent in forest soils (Paine, 1927) than in grassland soils where, particularly on acid soil types, *Penicillium* and *Trichoderma* are commonly reported. This relationship of species of *Mucor* with the forests is of some interest and may be not unconnected with the inability of these fungi to hydrolyse cellulose and with their ability to decompose protein (Abbott, 1923; Martin, Anderson & Goates, 1942). It is precisely in such situations that high protein content may be expected in the annual carpet of leaves deposited without previous undue destruction.

As well as the higher Basidiomycetes there are other striking omissions from the components of the fungal flora usually reported from soil. Species of *Pythium* may be considered. With the possible exception of Meredith (1940), and of the investigators mentioned above (p. 203), few workers in this field record species of the genus and yet they are commonly present in natural turf soils and are specially prolific in cultivated soils under vegetable crops. *Pythium ultimum* in an active state (Chesters, 1948) can be



isolated from such soils during all but the driest seasons of the year and it is almost true to say that *P. intermedium* is one of the most frequent of soil-inhabiting fungi. But all species of this genus prefer maize extract agar as their isolation substrate. Again *Rhizoctonia solani* (*Corticium vagum*) is not often recorded from pasture and from cultivated soils, yet its typical mycelium may be observed associated with plant debris in almost all soil rich in organic matter. It grows freely from debris on to agar, but will not often appear in dilution plates. Many other instances could be cited of similar omissions, and before leaving this matter I would draw your attention to the infrequent and irregular reports of species of the darker Hyphomycetes. Such fungi are present on plant debris in the upper layers of the soil and play no small part in the decomposition of structural material. They may perhaps be invaders, but some at least can be regularly obtained from precisely similar locations in the soil (e.g. *Helminthosporium biforme*, Mason & Hughes, in appendix to Chesters, 1948). It is possible that their absence is due to the difficulty of germinating detached spores or mycelial fragments such as would normally be present in suspensions. These difficulties are well known to those who attempt to grow species of the Dematiaceae collected above ground.

I should like to return for a few moments to the soil profile. Have we given enough thought to what goes on above the soil in relation to what we are striving to examine within the soil? The basic nutriment of fungi living in the soil ultimately derives from organic matter of plant or of animal origin—it may be the direct pabulum on which they live or it may supply the solutions on which free-living hyphae, if there are such, depend, and it almost certainly plays a part in the initial germination stages of spores in the soil. The plant fraction is obtained either from the aerial shoot systems or from the root systems. The latter, on death, and in so far as saprophytic fungi are concerned, are directly vulnerable to the fungus mycelium or to the fungus spores already present in the soil. The former present a very different state of affairs. The basal parts of the shoot system in contact with soil may be directly available to soil-inhabiting fungi but the distal parts are by no means thus exposed. They, on the contrary, are 'worked over' by weak parasitic and by saprophytic species; by a whole galaxy of Fungi Imperfecti, of Ascomycetes and even a few Hymenomycetes and they arrive at the soil surface very much depleted of their nutritional possibilities—they have lost much, but they have gained much which was foreign to their original make-up. Perhaps the one important exception to this state exists in deciduous trees and shrubs where the leaves reach the soil level little changed through prior fungal attack. Elsewhere air-borne saprophytes or weak parasites have utilized the easily available proteins and carbohydrates before stem or leaf reaches the soil and the continued growth of some of these species frequently depends on just this change of nutrition. From the point of view of the soil-inhabiting fungi such pretreatment means that cellulose complexes and less easily soluble nitrogen compounds, not forgetting the protoplasm and fungal cellulose of the mycelium of these vanguard fungi, is all that is left to the lot of the soil inhabitants. It is not to be wondered at that many of these actively



hydrolyse cellulose (Dubos, 1928; Jensen, H. L., 1931 *b*; Karnicka & Ziemiecka, 1935; Skinner, 1925; Vandecaveye, 1935); their power in this respect is linked with the availability of nitrogen, and Waksman has indicated that the ratio of cellulose decomposed to nitrogen utilized is in the region of 30:1 and that this ratio is relatively constant both in vitro and in field experiments. Since the ability to decompose cellulosic materials is closely linked with the availability of nitrogen, it is just at this point that the mucoraceous types are so important in soil as they are so predominantly the forms which liberate this nitrogen. To accomplish this effectively, not only proteins but also easily available carbohydrates are necessary—in nature they can be obtained from fresh or relatively fresh plant debris reaching the soil. If this argument is sound, it would explain why the species listed in diverse soil studies are confined to Mucoraceae and cellulose-decomposing Fungi Imperfecti and to such Ascomycetes as *Chaetomium globosum* and its allies. The occasional species of such lists may be derived from spores detached from mycelium of Fungi Imperfecti which is living in organic debris or spores which have been carried down from above. In the upper horizons of the soil such fungus-bearing debris occurs in quantity, and it is here that the filtering action of the soil is so manifest. While most studies of soil fungi have been conducted in these horizons the actual surface debris has frequently been carefully removed. I am certain that soil investigations in future must commence by examining this surface litter and must work downwards into the lower horizons. I agree that the task is difficult, that its danger lies in the inability to surface sterilize effectively, by currently accepted methods, portions of plant remains, and that selection in sampling must be the rule. Walker (1941) has already indicated that various surface sterilization methods affect the isolation results in no small degree. However, I am fully convinced that prolonged washing with renewed volumes of sterile detergent solution is probably just as effective in removing unwanted spores and other contaminants as is any treatment designed to kill chance spores. Combined with a careful scrutiny of the time of appearance of mycelial growths and with a careful selection of isolating media—not all of which need be agar—much useful information regarding the location of individual species, the grouping of species relative to units of material, the seasonal rise and fall of species during changes in the soil and the succession of individuals or groups of individuals, will be obtained. It is my view that through such studies we shall ultimately come upon the missing link in soil mycology—the elusive higher Basidiomycetes.

Within the more mineral portions of the soil similar considerations must be borne in mind. Moribund or dead roots reflect the same cycle of nutritional changes as the stem and leaf material, but the volume of nutrients is much reduced. A similar sequence appears to exist; mucoraceous types act as early colonists and are replaced by cellulose-destroying Hyphomycetes. The study of succession on root debris is probably one of the easier problems, as such debris can be effectively washed and yields results of some interest even on agar. Several investigators have noted the presence of species of *Mortierella* in the soil, and Bisby *et al.* (1935) go so far as to

state that, in the Canadian soil profiles studied by them, species of this genus proved to be the most abundant phycomycete. From the systematic accounts of the genus it is well established that many species occur on decaying debris on the soil surface. We have isolated a considerable number of strains of *Mortierella* from root debris, but their taxonomic treatment is a matter of some difficulty. It would appear, however, that there are relatively few species concerned. With experience in recognizing certain mycelial characteristics, it is possible to pick out strands of undoubted *Mortierella* hyphae extending from root debris into the soil and even to cultivate such strands on agar. There is reasonable certainty that some species of *Mortierella* occur on root debris as a regular habitat. Certain hyphae passing out from root fragments form vesicles in close proximity to the root tissue and such hyphae yield very fine, thread-like and sterile mycelium in agar culture. Whether these are also, in fact, species of *Mortierella* is open to doubt and one cannot help reflecting in this connexion on the observations of Butler (1939) regarding the arbuscular types of mycorrhizal fungi in plant roots.

To summarize what has been said regarding the examination of soils carrying natural vegetation; much of the past work deals with mass isolation from particular horizons in the soil; the source of cultured species is various—mycelium, freshly produced spores and dormant spores all participate; biochemical studies with such fungi indicate broad lines of succession related to the carbon-nitrogen requirements. It would appear that in the future, a new and profitable line of attack should be the consideration of the biology of species in relation to the plant debris on which they grow, followed by attempts to examine and interpret the micro-habitats of the fungi concerned.

Conditions are less stable in cultivated soils—the annual act of cultivation and the repeated husbandry to which they are subject, causes not only physical changes in structure, but also great variation in the type and the condition of the organic material added to the soil, and in the nitrogen and general salt content of the soil. It is evident that the addition of any form of green vegetation immediately stimulates the local development of fungal mycelium and that mucoraceous species in particular respond to this stimulus, when high protein content is concerned. Such localized and intense activity in a medium of relatively high nutrient value should bring into play the fullest possible effects of antagonism consistent with the food supply, and it has been repeatedly emphasized that antagonism and antibiosis are only really effective under such conditions—but a more detailed study of such phenomena is urgently necessary.

In arable soils the development of a crumb structure is of vital importance from the point of view of the physico-chemical and of the biological aspects (Erikson, 1947). It is here that the soil fungi play a most important part. Mycelium passing through the soil helps to bind the mineral fractions into aggregates (Waksman & Martin, 1939) which tend to resist leaching effects of percolating water, and which improve the aeration of the soil and the ease of root traverse through the soil. Some interesting investigations on this aspect have been reported. The actual degree of stability of the

crumb structure appears to be influenced to a varying degree by different moulds, when these are actively growing in the soil. Under definite experimental conditions Martin & Anderson (1942) have shown that, in silt and in sandy loam, the following series of increasing efficiency could be determined in relation to water stability of the aggregates:

Silt loam	Sandy loam
<i>Rhizopus nigricans</i>	<i>Rhizopus nigricans</i>
<i>Penicillium glaucum</i>	<i>Penicillium glaucum</i>
<i>Mucor rouxi</i>	<i>Aspergillus minutis</i>
<i>Aspergillus minutis</i>	<i>Mucor rouxi</i>
<i>Cladosporium</i>	<i>Cladosporium</i>

They point out that the order of effectiveness in producing water-stable aggregates approximately follows the successional appearance of these fungi during decomposition: *Rhizopus*, *Mucor*, *Penicillium*, *Aspergillus* and *Cladosporium*. Kanivets (1939) has demonstrated that aggregation at foci which develop in relation to micro-organisms in rhizosphere soil may be three or four times as great as aggregation in surrounding soil. *Trichoderma lignorum* appears to be particularly common in such aggregates, and artificial inoculation of soil with this species increased soil aggregation in the rhizospheres of cereals, pulses and root crops (Kanivets, Chariton & Tultshinskaya, 1940). Aggregation foci contained more free nitrogen, phosphorus and potassium than neighbouring soil (Kanivets, 1939). It would appear, therefore, that besides acting as centres for the development of crumb structure such local mycelial activity increases the available mineral constituents of the soil.

Study of the succession of fungal species, relative to plant debris in arable soils has an obvious bearing in relation to the species which are suspected of existing in a prolonged saprophytic phase in the soil. Such investigations may prove of particular interest with respect to species of *Phytophthora*. Although certain of these have been shown to be capable of living saprophytically in steamed soil (de Bruyn, 1922) there is no very clear evidence for or against the persistence of such mycelia in normal soil. I believe that the study of the fungal flora of plant debris in arable soil as it passes into ley may be of much interest and value in relation to the carry-over of specific pathogenic fungi from one crop to its next appearance in a rotation.

The fungal population of arable and natural soils has another important facet which requires much more extensive and intensive study than it has yet been afforded. Sachs and Knop in the later half of last century demonstrated that roots excrete materials into the external medium as well as absorbing salts from this medium. This encouraged Hiltner, in 1904, to suggest that the soil round roots should provide a good growing medium for micro-organisms, and to prove that micro-organisms were more numerous there than at other points in the soil. He called that area of soil which surrounded a root and received root exudates, the rhizosphere. The obvious importance of what is happening immediately external to the root tissues and to the root hairs need not be emphasized—but it should be realised that two very different sets of problems are inter-related at this

point: root action on the soil population and soil population effect directly on the root as the absorbing organ of the plant. Starkey (1929) initiated the modern study of the rhizosphere population and demonstrated that for adult plants the fungal fraction of the soil population was less affected by the presence of living roots than was either the bacterial or the actinomycete population, but that all three were more numerous in the rhizosphere than in the 'open' soil. This work was carried out by dilution-plating methods and by virtue of that fact suffered initial inaccuracies. He demonstrated also that the maximum effect was evident in late stages of growth or during flowering and that to a certain extent plants of different species did not stimulate the soil micro-flora to the same extent. While the difference in estimated fungal population between rhizosphere soil and soil remote from roots was found to be not fully significant, the difference between the estimated fungal population on the surface tissues of roots and in the surrounding soil was marked (Starkey, 1931*a*; Thom & Humfeld, 1932). It would appear that the soil type has some influence on the degree of difference exhibited between the rhizosphere population and the population of open soil, and Adati (1939) has shown that the difference is greater in sand than in soil rich in humus. This result would support the theory that root excretions act as growth stimulants, because these are more likely to be potent in soils poor in other forms of nutriment.

The introduction of the Rossi-Cholodny slide technique provided a further method of examining and of estimating the population of the rhizosphere. By burying slides in relation to growing roots Starkey (1938) demonstrated the presence of fungal hyphae close to root hairs and to small roots. It was clear that while both living and dead roots bore such fungal associates, they were more numerous on the latter, where hyphae and spores were seen. Infection of living roots was also observed. In general, the hyphae within the rhizosphere of living roots did not sporulate, but in the open soil sporulation was observed on mycelium associated with minute particles of organic matter. Starkey comments on the occurrence of bacteria on the surface of living fungal hyphae, forming either diffuse patches over the wall or local aggregations. More numerous bacterial accumulations were evident on dead mycelium. That the bacterial colonies on living hyphae are probably related to the additional soluble materials in this region is highly possible. That the increases in bacterial numbers in rhizospheres may in part reflect the stimulation of bacterial growth following the death of fungal mycelium must not be overlooked. Timonin (1940*a*) concludes that in the presence of young roots bacterial development is enhanced as compared with fungal development, and makes the very pertinent remark that this stimulation is probably in the mycelial condition and that free sporulation occurs more readily in open soil—perhaps in relation to the lower moisture content. Timonin has demonstrated the interesting fact that the rhizosphere population of individuals of the same variety of a flowering species exhibited little change when the soils in which the plants were growing had been subject to varying fertilizer treatments which themselves resulted in differences in crop yields. This is at variance with observations elsewhere in open soils.

There, addition of organic and of inorganic fertilizers stimulates the development of the soil micro-flora, including the soil fungi. It appears, therefore, that so far as the rhizosphere is concerned, the type of crop is more important than the fertilizer treatment. This may be interpreted as indicating the essential relationship of the rhizosphere population to the total metabolites of the region. In this connexion it is interesting to realize that Timonin (1940 *b*; cf. West & Lochhead, 1940) found the population of the rhizosphere of a flax susceptible to wilt to be higher than that of a flax resistant to wilt, and a similar relationship was demonstrated in varieties of tobacco, some susceptible and some resistant to black root rot. This confirms the similar reports of Thom and Humfeld (1932).

During earlier studies of soil populations in Manitoba, Timonin (1935) had demonstrated higher counts from soils below the wilting-point for flowering plants than from moist soils easily supporting vegetation, and in his studies on rhizosphere populations he drew attention to the fact that in controlled experiments rhizosphere populations were higher in soils of low moisture content. His explanation of this is worthy of notice, as he points out that the soil solution is more likely to be concentrated in soil at 30 % of its total moisture holding than in soil at 60 % and that the Chlodny slide technique demonstrated increased root-hair development in the drier soil. This larger absorbing surface, besides releasing additional nutriment by death of the older root hairs, is likely to increase the accumulation of the excreted materials into the rhizosphere and hence stimulate microbial development. Even in almost sterile, arid soils a strong rhizosphere population exists (Sabinin & Minina, 1932).

This important aspect of soil mycology must necessarily expand in future years when new methods of attack are devised and when much more is known about the physiology of the soil-inhabiting fungi. So far, the attack has been aimed at estimating the quantity of the total rhizosphere population and of the fraction of it which can be cultivated on agar plates; at inspecting small areas of this rhizosphere population as it adheres to slide traps inserted to apprehend it; and at demonstrating the effect of the growing plant roots on these aspects of the rhizosphere. Very little has been written of the effect of the rhizosphere population or its fungal fraction upon the normal healthy flowering plant. I am well aware of the extensive literature relating to the mycorrhizal habit and of the voluminous researches on the intimate relationships between root-attacking fungi, the soil micro-flora and the host root, but this field is beyond the path I have chosen to follow for the moment. A study of the part played by the rhizosphere in determining the soil solution available to the normal plant root is of much importance, and already studies are indicating that the availability of phosphates to the plant root may owe much to the action of the fungal portion of the rhizosphere. The production of nitrogenous and carbohydrate units by the soil fungi associated with the rhizosphere and their relationship to plant growth is likely to prove of interest, and it may well be that the flowering plant is shown to be far more heterotrophic than has been suspected, when a more complete analysis of the role of the soil microflora—and not the least, of the fungal part of that flora—has been made.

Thom and Humfeld (1932) have pointed out that the rhizosphere of maize roots gave equally high fungal counts in acid or in alkaline soils, and have inferred that the lack of significant variation of these numbers is due to the fact that the root zone carries its own pH value irrespective of the soil through which it passes, and that this zone is in the region between pH 6 and 7.5. The fungal population of such rhizospheres consisted predominantly of species of *Trichoderma* in acid soil and of *Penicillium luteum* and other species of this genus in alkaline soil. Melin (1934) has shown that roots secrete phosphatides and that these stimulate the development of soil-inhabiting fungi. It may well be (as D'Aeth has suggested, 1939) that this is a further potent factor in the relationship of the rhizosphere fungi and the plant roots. Jahn (1934) includes what are usually considered as rhizosphere fungi in his group of peritrophic mycorrhiza, stating that although they do not penetrate the root tissues they have a symbiotic function. He points out that Friesleben (1934) had already shown for *Vaccinium* that the addition of *Penicillium glaucum* (*sic*) to pure culture seedlings of the host produced a stimulation of the higher plant of similar order to that of a proper mycorrhizal partner. Jahn believes that the valuable contribution of the peritrophic fungi lies in the fact that they develop a zone of high acidity round their associated roots, and that such species as *Mucor racemosus* and *M. rammanianus* have strains which are confined to these root zones. According to him the characteristic fungi in such locations are species of *Penicillium* and *Mucor*. In later studies, Jahn (1936) expands this conception to all classes of mycorrhizal fungi (endo-, ecto-, and peritrophic). He believes their value lies in their ability to render available the bound salts of the soil by virtue of the production of an acid medium round the roots. It is probable that, particularly in relation to phosphorus availability (Abbott, 1923), this aspect of the rhizosphere will prove to be of great importance.

I should like to close by indicating the lines which appear to me to hold promise of future developments in the study of soil-inhabiting fungi. Foremost of these is an investigation of the crude organic matter at and immediately within the soil surface—I am certain that it is within this zone that the secret of the higher Basidiomycetes will be uncovered. I am equally certain that this can only be adequately pursued alongside pure culture studies of isolations from the sporophores of named species growing at the point of investigation.

Investigation of the space relationship of mycelium in the soil with reference to organic debris may lead us to modify the very useful conception of inhabitant and invader species to a concept of local and wide colonizers, and discover to us that the inhabitant flora consists of many more members which are constant in soil, but which, heretofore, have not been able to compete at isolation with the wide colonizers such as species of *Mucor* and their allies.

Development of methods of investigating the immediate surface of contact between soil and root must take place, and a united endeavour between the mycologist, the plant pathologist and the plant physiologist in this field is long overdue and of the utmost importance in understanding plant economy.



Elucidation of the natural effects of antibiosis, if such exist, of its whys and wherefores in the day-to-day life of the soil population must be seriously undertaken alongside the quite different and equally vital study of antibiotic action in human and animal affairs.

Work on the physiology of soil fungi, on their relationship to other members of the soil population, on their vital place in the physics of arable soils and on their economic effects in crop production are all ultimately based not only on relative quantities at work but also, and, I would plead, more cogently, on the precise individuals operating at one time. This knowledge cannot come without detailed and painstaking study of the taxonomy of soil-inhabiting fungi. This, in turn, is a matter for pure culture investigation *in vitro*, if not necessarily in agar-agar, and it may frequently require a deep appreciation of the intimate physiology of the species in question, as the excellent investigations of Melin and his school have demonstrated. The taxonomist working on soil fungi not only must be prepared to identify his species in a culture tube, without knowing what he put into it, but also must be capable of persuading the species to deliver up the criteria by which it may be known—in other words, must be capable of making it spore. New methods and new outlooks are necessary to study this fundamental field in which the fungus plays a vital part in the medium from which the essences of life in this world derive.

I wish to conclude by quoting from R. L. Stevenson a passage whose wide application to botany was first brought to my notice as an undergraduate student, and whose immediate application to the investigation of fungi inhabiting soil is patent: 'For to travel hopefully is a better thing than to arrive, and the true success is to labour.'

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## A STUDY OF VIOLET ROOT ROT

II. EFFECT OF SUBSTRATUM ON SURVIVAL OF  
*HELICOBASIDIUM PURPUREUM* COLONIES  
IN THE SOILBy S. D. GARRETT, *Rothamsted Experimental Station, Harpenden*

It has been assumed, though so far without experimental demonstration, that sclerotia of *Helicobasidium purpureum* Pat. are responsible for prolonged survival of this fungus in the soil from one host crop to the next (Hull & Wilson, 1946). The sclerotia have been described by Duhamel (1728), Duggar (1915), Faris (1921), Hull and Wilson (1946) and Garrett (1946a). In the present paper are described some experiments on the survival of colonies of *H. purpureum* on different agar substrata, when buried in jars of soil in the laboratory. This work was planned as a preliminary to a projected study of the production of sclerotia on the host plant, and of their survival in the soil. Although this work is incomplete, circumstances prevented its continuation, and so the results are presented.

## METHOD

Sclerotia can be observed in pure culture on suitable nutrient agars, and, when mature, can be picked off the plates for purposes of investigation; for work on a large scale, however, this method has serious disadvantages. Sclerotia are formed at different distances from the centre of the colony, and to this difference in position is to be added a great variation in size of individual sclerotia. Individual sclerotia, moreover, may fuse to form compound sclerotial masses, and it is difficult to detach sclerotia cleanly and without wounding from the substrate. It was therefore decided to use, not individual sclerotia, but individual small colonies of the fungus in this preliminary study of survival. Briefly, the method consisted in the inoculation of agar plates (9 cm. diameter) at ten equidistant points, 14 mm. from each other and 9 mm. from the margin of the plate. Inoculum disks, of diameter 4 mm., were cut out marginally from colonies on double strength meat-malt agar (for composition, see below), with a sterile no. 1 size cork borer. The sites for inoculation were marked on the bottom of each dish with the aid of a stencil, and the inoculation carried out with the ten-point inoculating needle already described (Garrett, 1946b). The plates were incubated for two months at 25° C., and the ten colonies were then cut out of each plate and buried in 2 lb. glass jam jars of soil. The soil employed in all the experiments was a mixture of one part (by volume) of Harpenden allotment soil with three parts of sand, and was maintained at a moisture content of 50 % saturation; depth of soil below the colonies was 1 in., and above, 2 in. Methods of preparing and handling soil were described in the preceding paper (Garrett, 1946a). After three to four

months in the soil, the ten colonies were washed free of soil over a small 3 mm. sieve, and tested for viability by inoculation of carrot seedlings grown in the glasshouse.

For this purpose, stump-rooted carrot seedlings were grown in eleven equidistantly spaced groups of four plants each in wooden flats ( $14 \times 8\frac{1}{2} \times 3$  in.) of the same 1 soil : 3 sand mixture as used in the jars. This soil/sand mixture was known to be more favourable for growth of the mycelial strands of *H. purpureum* than undiluted soil (Garrett, 1946a), and a light-textured soil is generally considered to be specially favourable for growth of carrots. The planting of eleven groups of seedlings per box gave one group as a spare, and also permitted a more symmetrical arrangement of groups within the box. Seed of Early Market stump-rooted carrot was sown around the periphery of circular depressions 1 in. in diameter, and seedlings were thinned to four per group after three weeks. Sodium nitrate and potassium phosphate were applied in 100 ml. water per box, each at the rate of 2 cwt. per acre (1.86 g. per box) at three and seven weeks after sowing. Groups of seedlings were inoculated with the colonies of *H. purpureum* to be tested at the age of one month; a vertical slit wide enough to receive the colony was made in the soil in the centre of each group of seedlings, and closed up again after insertion of the colony. After inoculation, the seedlings were allowed to grow for another two months, and were then washed free of soil for examination of the tap roots; the number of colonies (out of ten) that had produced visible infection was recorded for each box. The lesions produced by *H. purpureum*, with their characteristic infection cushions, were unmistakable even to the naked eye; frequently, the whole of the tap root was infected.

#### EXPERIMENTAL

*Experiment I.* An attempt was made to determine the optimum period for incubation of colonies on the agar plates at 25° C. The three different agar media employed were variations of the meat-malt agar found by Buddin and Wakefield (1924) to be the best of any that they tested for growth of *H. purpureum*. The standard formula taken for this agar was as follows: Lemco beef extract, 10 g.; malt extract, 30 g.; agar, 20 g.; and distilled water, 1 l. In this experiment, the three agars chosen for comparison were malt agar (0 meat : 1 malt), normal strength meat-malt agar (1 meat : 1 malt), and double strength meat-malt agar (2 meat : 2 malt). Each agar was poured both 3 mm. deep (20 ml. per plate) and 6 mm. deep (40 ml. per plate), making six comparisons in all. After inoculation with the ten-point inoculating needle, plates were incubated at 25° C. for periods of three, six, nine and fourteen weeks; after each interval, two plates were taken from each of the six series for inoculation of the test carrot seedlings.

Percentages of viable colonies at each test are shown in Table 1. The results indicate that a decline in viability of colonies occurs when they are incubated at 25° C. for a period exceeding nine weeks. An incubation period of two months at 25° C. was therefore selected as a standard for further experiments. No great or consistent increase in viability was

secured by pouring the agar at double depth, so that the standard depth of 3 mm. was selected for further experiments.

*Experiment II.* Colonies were grown on malt agar and on meat-malt agar for two months at 25° C., and were then buried in soil for periods of three and nine months before testing on carrot seedlings. Four jars, i.e. forty colonies, were taken as a sample from each series to be tested; results are shown in Table 2.

The difference in survival of colonies on malt and meat-malt agars is very pronounced, and is consistent with observations made when colonies were recovered from the soil, preparatory to inoculation of carrots. The

Table 1. *Percentage viable colonies*

Type and depth of agar	Age of colonies (weeks)			
	3	6	9	14
0 meat:1 malt, shallow	50	90	90	40
0 meat:1 malt, deep	75	90	100	70*
1 meat:1 malt, shallow	85	85	75	5
1 meat:1 malt, deep	95	100	100	10
2 meat:2 malt, shallow	100	100	95	60
2 meat:2 malt, deep	100	100	80	60

\* 10 colonies only in test

Table 2. *Percentage viable colonies*

	Period of burial in soil (months)	
	3	9
Malt agar	75	68
Meat-malt agar	8	—

Table 3. *Percentage viable colonies*

Concentration of malt in agar	Ratio of meat:malt in agar		
	0:1	1:1	2:1
Half strength	18	8	0
Normal strength	93	5	13
Double strength	98	0	0

colonies on meat-malt agar consisted of mycelial mats, with relatively small and undeveloped sclerotia. In the colonies on malt agar, the central inoculum disk usually carried one or more large sclerotia, or a sclerotial aggregate, firm and resilient in texture.

*Experiment III.* In this experiment, three different ratios of meat to malt constituent were tested, viz. 0 meat:1 malt, 1 meat:1 malt, and 2 meat:1 malt (1 meat:1 malt representing the standard ratio of 1 g. meat to 3 g. malt, and not equal parts by weight). Each ratio was made up at three different malt concentrations, viz. half normal, normal and twice normal strengths. After incubation of the inoculated plates at 25° C. for two months, colonies were buried in soil and left there for four months, after which period forty colonies from each series were tested by inoculation of carrot seedlings (Table 3).



Results again show that addition of meat extract to the agar greatly reduced percentage survival of colonies. Reduction of malt agar to half strength also markedly reduced percentage survival. These results are again consistent with the appearance of colonies at time of recovery from the soil. In those on malt agar, firm and resilient sclerotia, or sclerotial masses, had developed around the inoculum disk, this development being greatest on the double strength malt agar. Few or small sclerotia were observed on the meat-malt agars. Persistence of the mycelial felt was much better on the 1 meat:1 malt agars than on the 2 meat:1 malt agars. On the double strength 2 meat:1 malt agar (i.e. 4 meat:2 malt), the mycelial mats had disintegrated to a considerable extent in three out of the four soil jars.

A possible interpretation of these results is suggested by the observation that addition of meat extract (nitrogen content 8.85 %) to the agar increases the density of mycelial growth. This must occur at the expense of the carbohydrate reserves required for sclerotial production by the fungus colony. Moreover, when the colony is buried in the soil, the excess of nitrogen must encourage the growth of soil micro-organisms, which will then deplete still further the reserves of carbohydrate available for *H. purpureum* in the agar substrate.

*Experiment IV.* In this experiment, half strength malt agar (i.e. 1.5 % malt extract), with and without the addition of 1 % dextrose, was made up with 0, 0.125, 0.25 and 0.5 % sodium nitrate. Ten plates of each of the eight agars were inoculated in the usual way, and incubated for two months at 25° C. Colonies were then buried in soil for sixteen weeks, after which period ninety colonies from each of six series (two series were omitted from the test) were tested by inoculation of carrot seedlings (Table 4).

Table 4. *Percentage viable colonies*

	Percentage NaNO <sub>3</sub> in agar			
	0	0.125	0.25	0.5
1.5 % malt + 1 % dextrose	42	4	0	13
1.5 % malt alone	11	4	—	—

These results show that both omission of dextrose and addition of sodium nitrate have depressed survival of *H. purpureum*, and therefore support the interpretation placed upon the results of Exp. III. Once again the results of this experiment could have been forecast, very approximately, from the appearance of the colonies at time of recovery from the soil. Colonies on 1.5 % malt + 1 % dextrose showed the best development of firm sclerotia, those on 1.5 % malt without dextrose being much poorer. Few or no sclerotia were formed on agars to which sodium nitrate had been added. The maximum survival of colonies in this experiment was only 42 %, on 1.5 % malt + 1 % dextrose; this figure is intermediate between that on 1.5 % malt (18) and that on 3 % malt (93) in Exp. III.

*Experiment V.* This experiment was designed to investigate the effect of a wider range of carbohydrate and nitrate nitrogen concentrations than

that employed in Exp. IV. The basal medium employed was as follows: malt extract, 15 g.; potassium phosphate, 1 g.; magnesium sulphate (crystalline), 0.5 g.; agar, 20 g.; and distilled water, 1 l. Malt extract was assumed to supply all possibly essential growth factors (the growth-factor requirements of *H. purpureum* have not been determined). The mineral nutrients were added to ensure that growth of the fungus was not limited by nutrients other than carbohydrate and nitrogen. To samples of the basal medium were added, respectively, 1, 2 and 4 % dextrose. Each of the three dextrose series was made up into six subordinate series, with the following percentages of nitrogen as sodium nitrate: 0, 0.01, 0.02, 0.04, 0.08 and 0.16 (with the single exception that the series 1 % dextrose + 0.16 % nitrogen was omitted, as likely to lie well beyond the range of interest). Three plates were poured with each of the seventeen agars, inoculated, and incubated for eight weeks at 25° C. The thirty colonies of each series were next buried in soil for a period of two months, and then tested for viability by inoculation of carrot seedlings. Unfortunately it was impossible to allow colonies to remain in the soil for the longer period of three to four months employed in the previous experiments. The experiment was not started until 29 May, and the carrots were inoculated on 22-24 September; had inoculation been delayed much longer, the carrots would not have made sufficiently good growth under the deteriorating light conditions. Results are given in Table 5.

Table 5. *Percentage viable colonies*

Percentage nitrogen	Percentage supplementary dextrose		
	4	2	1
0	37	37	16
0.01	73	30	50
0.02	50	40	23
0.04	47	53	17
0.08	73	13	3
0.16	70	30	—

Inspection of these results shows that the optimum level of nitrogen for survival of colonies increases with the concentration of dextrose: it is 0.01 % nitrogen for 1 % dextrose, 0.04 % nitrogen for 2 % dextrose, and extends up to 0.16 % nitrogen (the highest level included in the experiment) for 4 % dextrose. These figures support the view, advanced above, that the depressing effect of nitrogen upon colony survival is due to more rapid consumption of available carbohydrate by the denser mycelial growth, induced by a higher nitrogen content of the medium. These figures are also quite consistent with those given in Tables 2 and 3, showing that percentage colony survival was low on meat-malt agar, both at normal and at double strengths. An approximate calculation shows that, to secure a ratio of nitrogen to carbohydrate equivalent to that in meat-malt agar, it would be necessary to supply 0.10 % nitrogen to the 1 % dextrose series, 0.15 % nitrogen to the 2 % dextrose series, and 0.25 % nitrogen to the 4 % dextrose series (the malt extract contains 73 % dry matter, and 0.85 % nitrogen on a wet basis: the meat extract contains 8.85 % nitrogen on a wet basis).

Caution is obviously necessary in drawing conclusions from an unfinished investigation on the survival of *H. purpureum* colonies on an agar substratum. The observed effects of increased carbohydrate concentration (both in the form of malt extract and of dextrose) in increasing survival, and of nitrogen (both as meat extract and as sodium nitrate) in reducing it, may be tentatively ascribed to their visible effects on the formation of sclerotia. This phenomenon, however, is quite possibly a laboratory artefact, without parallel in the field. On the culture plate, the carbohydrate reserves, whether in the form of malt extract or of dextrose, are readily available, and may therefore be rapidly consumed in the early stages of colony growth, provided that adequate supplies of nitrogen and other essential nutrients are present. On the host plant, on the other hand, the food reserves open to the fungus must usually be adequate for formation of viable sclerotia, for the very reason that the developing sclerotia can probably continue to draw on those food reserves that are not too readily available, and therefore cannot be exhausted during the earlier phases of mycelial growth.

A possible parallel to these results is afforded by the observations of Geach (1936) working with cultures of *Aphanomyces euteiches*, the causal agent of pea root rot. Geach found that on 2 % cornmeal agar, mycelial growth was sparse, but production of oospores was abundant; on 2 % cornmeal + 2 % peptone agar, on the other hand, mycelial growth was dense, but no oospores were produced. In the absence of oospores, cultures lost their viability rather rapidly, and no growth was made by transfers from cultures thirty-three days old. Oospore formation was also inhibited by the addition to 2 % cornmeal agar of 0.4 % ammonium sulphate or of 0.5 % sodium nitrate.

#### SUMMARY

Plates of different nutrient agars were inoculated at ten equidistant points with *Helicobasidium purpureum* and incubated for two months at 25° C.; the fungus colonies were buried in jars of soil for three to four months, and then tested for viability by inoculation of carrot seedlings. Survival was increased by raising the carbohydrate concentration of the medium, but depressed by excess of nitrogen. The optimum nitrogen requirement for survival increased with rise in carbohydrate content of the medium. Survival of colonies was correlated with the production of firm resilient sclerotia around the centre of the colony. The depressing effect of excess nitrogen upon production of sclerotia and survival of colonies is attributed to an increased density of mycelial growth, leading to reduction of carbohydrate level below that required for maturation of viable sclerotia.

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# THE MORPHOLOGY OF *PENICILLIUM CHRYSOGENUM* IN SUBMERGED FERMENTATIONS

By R. B. DUCKWORTH AND G. C. M. HARRIS

*Imperial Chemical Industries Ltd., Biological Laboratories,  
Hexagon House, Manchester 9*

(With Plates XVIII and XIX and 10 Text-figures)

## INTRODUCTION

Several papers have been written during the last few years on the morphology of the moulds *Penicillium notatum* and *P. chrysogenum* used for the production of penicillin. Of these the paper by Raper and Alexander (1945) describing the most important of the strains used is of particular interest. These workers, however, dealt almost exclusively with surface cultures of the moulds; the morphology of these organisms in submerged culture still awaits attention. The effects of agitation on the morphology of fungi were first investigated over fifty years ago, when Russell (1892) observed that such conditions favoured a bud-like rather than the more normal filamentous form. A few years later, Ray (1897) devoted a chapter in his thesis to the influence of agitation on *Sterigmatocystis alba* (*Aspergillus candidus* group). Of particular interest were his conclusions that: (a) the rate of growth in agitated culture is higher than in similar still cultures; (b) spherical colonies having a pseudoparenchymatous structure are formed; (c) atypical reproductive structures and a 'forme de conservation résistante' occur.

In 1908 Sartory produced a comprehensive thesis on the effect of agitation on species of the Mucorales, Ascomycetes and Fungi Imperfecti. He included three species of *Penicillium*. His investigation was more detailed than those previously undertaken and included sectioning of the colonies and the isolation and growth of various anomalous structures. While agreeing in the main with Ray he introduced certain new generalizations, namely, that (a) increase in the rate of agitation accentuates malformation of the hyphae and, in particular, budding; (b) the spherical form of colony is not universal; (c) colonies fragment; (d) abnormal forms, when grown in surface culture, pass through a series of stages back to the normal.

Kluyver and Perquin (1933) published a paper on *Aspergillus flavus* in which they emphasized the high metabolic rate obtained in agitated and aerated conditions. Little further work on the structure of submerged cultures was described until Burkholder and Sinnott (1945) published a general account of the morphology of a wide range of fungi which they grew in shaken culture. Among these were *Penicillium notatum* and *P. chrysogenum*. As was general under such conditions, globose colonies were obtained.

The importance of the type of culture produced is generally recognized by manufacturers of penicillin. Moyer and Coghill (1946) comment on the fact that certain types of growth are more favourable than others for the production of high yields of penicillin. This fact soon became apparent in the course of experimental work in our laboratories, and accordingly the morphology of *P. chrysogenum* under conditions of submerged fermentation has been made the subject of intensive investigation. In this communication the morphological changes which take place in typical cultures grown under standardized conditions are described and discussed.

#### EXPERIMENTAL METHODS

Commercially, it has generally been found advantageous to carry out the penicillin fermentation in two stages. In the first stage, conidia of the mould are grown in a medium containing glucose and corn-steep liquor to produce a mycelial inoculum. In the second stage, this mycelial inoculum is transferred to a medium containing lactose and corn-steep liquor (the penicillin fermentation).

*The organism.* The culture used was derived from a strain of *P. chrysogenum*, Q 176, which was prepared at the University of Wisconsin, Madison, Wisconsin.

*Media.* The media used were as follows (all percentages are w/v):

(a) Mycelial inoculum:	%	(b) Penicillin fermentation:	%
Glucose chips	6	Lactose B.P.	2
Corn-steep-liquor solids	2	Corn-steep-liquor solids	2
Chalk	0.5	Chalk	0.5
Water to	100	Phenylacetic acid	0.15*
		Water to	100

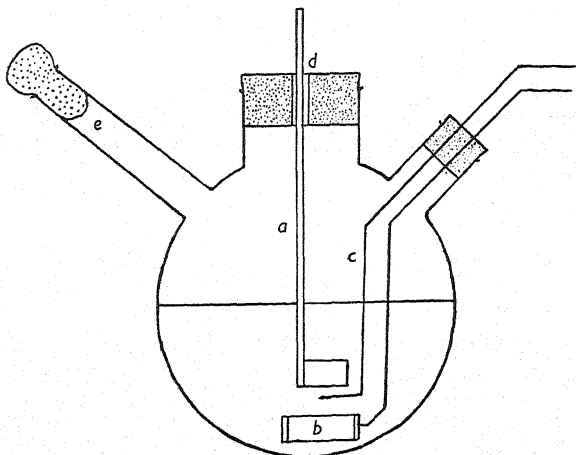
\* Added in three lots at 0, 24 and 48 hr., dissolved in the theoretical quantity of sodium hydroxide solution.

*The Mycelial inoculum.* 750 ml. conical flasks containing 100 ml. of medium were inoculated with  $10^8$  conidia of the mould. The conidia were grown on a glycerol-molasses-peptone-agar medium (cf. Frank, Calam & Gregory, 1948), suspended in water, and their concentration estimated by means of a haemocytometer. The inoculated flasks were incubated at 23–25° C. on a shaking machine which imparted to each flask a rotary motion. Each flask completed 100 cycles of 3 in. diameter per minute.

*The Penicillin fermentation.* Two-litre 'Pyrex' bolt-head flasks fitted with a single-bladed paddle agitator ( $\frac{3}{4}$  in. high by  $1\frac{1}{2}$  in. long) rotating at 600–650 r.p.m. were used (Text-fig. 1). The stirrer-shaft entered the neck of the flask by means of a gland designed to exclude micro-organisms. Additional provision was made for the admission of an air-pipe, for inoculation and for sampling. Air was passed in at the rate of 1.2 l./min., and was dispersed by a length of porous tubing (Aerox Ltd., P 28, 1 in. long,  $\frac{7}{8}$  in. diameter,  $\frac{1}{2}$  in. bore). The flasks each contained 1.1 l. of medium. Excessive foaming was prevented by the addition, from time to time, of arachis oil containing 3 % octadecanol.

Penicillin fermentations were inoculated by the addition of a forty-eight-hour mycelial inoculum (100 ml.) prepared as described above.

*Sampling and preparation of specimens.* Five-millilitre samples were taken from the cultures at frequent intervals and fixed in 10 % formalin. Slide preparations were stained with cotton blue in lactophenol.



Text-fig. 1. Bolthead culture vessel used for laboratory scale penicillin fermentations. *a*, flag stirrer. *b*, porous tube to distribute incoming air. *c*, tube for sampling. *d*, bearing designed to exclude micro-organisms. *e*, tube for inoculation and outlet for air.

#### HYPHAL DEVELOPMENT IN THE MYCELIAL INOCULUM MEDIUM

##### *Germination of conidia*

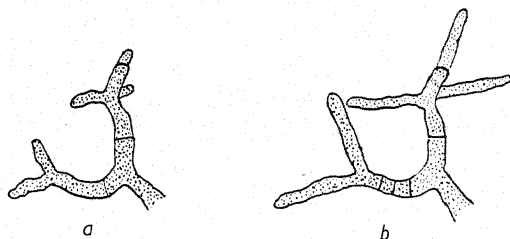
When suspended in water the conidia of *Penicillium chrysogenum*, Q 176, vary in diameter from  $3.2$  to  $8.3 \mu$ , averaging  $4.6 \mu$ . Thom (1930) found occasional abnormally large conidia in many species of *Penicillium*. These were so rare that he ignored them when giving the range of conidial size. In our suspensions these large conidia occur to the extent of about 0.5 %. In agitated medium the conidia begin to swell. This swelling ceases temporarily at the time of germination, at which stage most of them are between  $5$  and  $7 \mu$  in diameter, though the original abnormally large conidia are naturally greater in diameter. Swelling of this order for conidia germinating on agar media has been mentioned by Thom (1930) in *Penicillium* spp. generally and by Dr P. W. Brian (private communication) in *P. expansum*. Dr Brian also states that this swelling ceases when percentage germination has reached a high level.

In our conditions, 50 % germination occurs consistently between the seventh and eighth hour (Davies, Duckworth & Harris, 1948) and by the twelfth hour about 95 % of the conidia have germinated. Grenfell, Legge & White (1947) estimated that only about 45 % of the conidia, which they were using to inoculate penicillin fermentations, were viable. The discrepancy is probably accounted for by the different methods of assessment employed, since the plating method used by these workers would probably give low results unless multiconidial colonies, loss of conidia during transfer and desiccation of spores were avoided.



Germination of conidia of the *Penicillium notatum-chrysogenum* group within agitated media appears to be more rapid than on the surface of solid or liquid media (Baldwin & Harris, unpublished).

The conidia in the original suspension are separate but they soon become attached in groups. The effect of grouping on the organization of the mycelium, for example into spherical colonies, will be discussed in a later communication. Most of the conidia are spherical and germinate by the formation of a protuberance which rapidly increases in length to form a germ-tube  $4-5\mu$  wide. This tube stains more rapidly than either the parent conidium or other ungerminated conidia. At the time of germination almost all the conidia produce one germ-tube only, but occasionally two or very rarely three or four tubes are produced simultaneously. It is usual for multiple germ-tubes to grow out equidistant from each other in the same plane. Cytological changes occurring at the time of germination are being investigated.



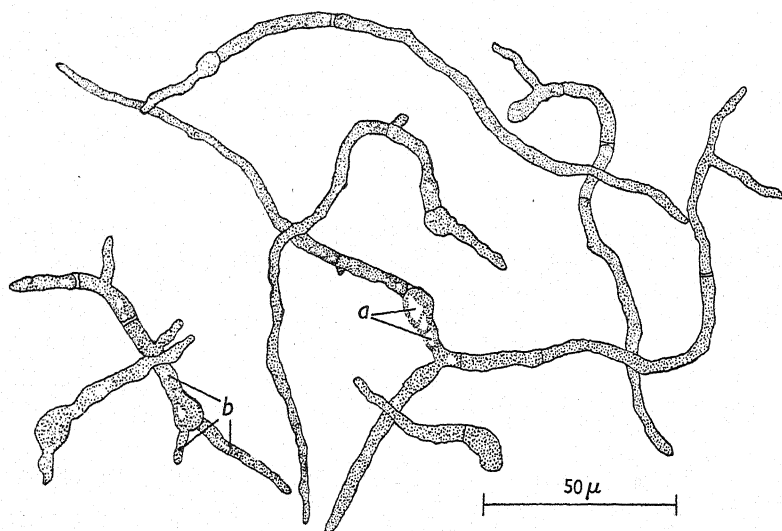
Text-fig. 2. Region of growth. *a*, part of a growing colony of *Penicillium chrysogenum*, Q 176. Notice position of septa. *b*, the same colony three hours later, showing growth of the apical regions only.

*Normal hyphae.* Growth is confined to the terminal 'cells' of the original germ-tubes and of side branches (Text-fig. 2). Two or three per cent of the germ-tubes have produced a branch by the twelfth hour, and by the fifteenth hour branches are common (Text-fig. 3 and Pl. XVIII, fig. 1). Later they are produced at irregular intervals along the parent hypha. Each branch normally arises from a region just behind a septum and is set at an angle of  $70-90^\circ$  to the direction of growth of the parent hypha.

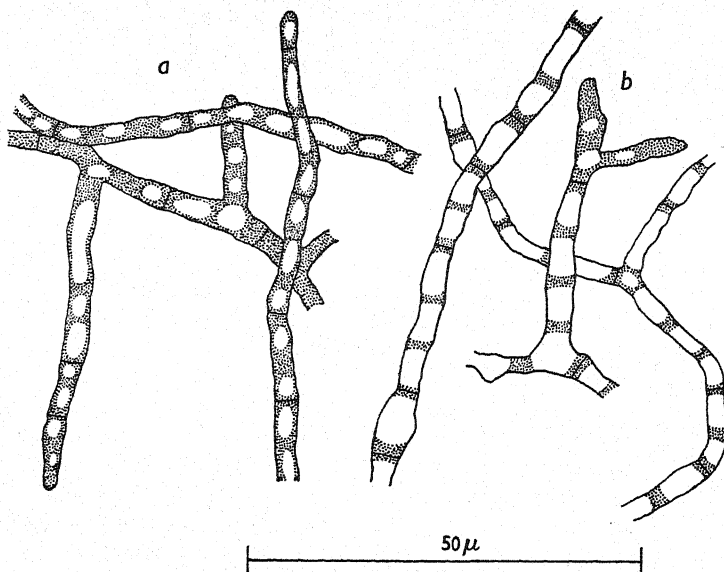
Most of the hyphae in the young culture elongate at a rate of about  $30\mu$  per hour, though, when germ-tubes are formed serially from a single conidium, the later ones frequently do not finally exceed  $15\mu$  in length.

Very small vacuoles can be seen in some germ-tubes at twelve hours. These vacuoles, which later increase in size and frequency, are usually oval in outline and appear as clear, sharply defined areas in the densely staining cytoplasm (Text-fig. 4*a*, 'chain vacuolation'). A rapid increase in vacuolation occurs between the twenty-fourth and thirtieth hours, and during this period short regions between septa become completely devoid of cytoplasm.

It seems probable that the formation of chains of oval vacuoles is a stage in the normal maturation process of this organism. A further stage in the process, known but seldom seen in the inoculum culture, is shown by an



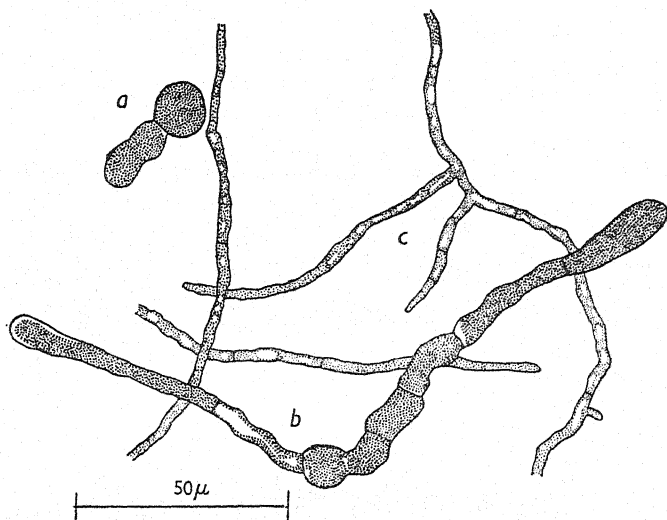
Text-fig. 3. Hyphal appearance at the fifteenth hour in the inoculum medium. *a*, vacuoles. *b*, three germ-tubes arising from one spore.



Text-fig. 4. *a*, 'chain vacuolation'. Rows of round or oval vacuoles giving a characteristic appearance. *b*, 'ladder vacuolation'. The vacuoles are larger and fill entire regions of the hyphae.

increase in the size of the vacuoles so that they almost completely fill the hyphae (Text-fig. 4*b* 'ladder vacuolation'). More commonly the unvacuolated or 'chain-vacuolated' contents withdraw from the walls and finally disappear.

The width of the young hyphal regions just behind the tip decreases during the course of the fermentation. This is noticeable at the eighteenth hour (*c.*  $3.5\mu$ ), is very obvious between the twenty-first and forty-eighth hour in the mycelial inoculum medium (*c.*  $2.5\mu$ ) and, as will be seen later, is still more pronounced in the penicillin fermentation (*c.*  $1.5\mu$ ). Branches produced from the younger regions of the hyphae are themselves narrower, less frequent and usually set at a smaller angle to the direction of growth of the parent hypha, than are those formed earlier.



Text-fig. 5. Late-germinating spores, producing wide densely staining outgrowths at the thirtieth hour in the inoculum medium. *a*, newly germinated swollen spore. *b*, one which has produced two germ tubes. Notice the withdrawal of the hyphal contents and the typical clavate tips. *c*, normal hyphae showing considerable vacuolation.

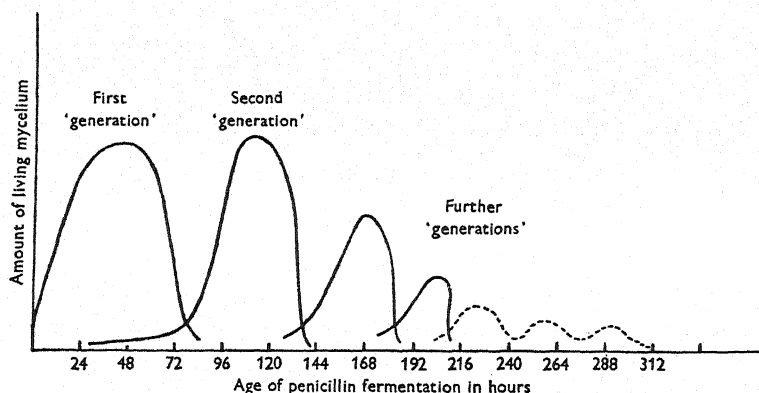
*Other hyphal forms.* In addition to the normal hyphae, certain hitherto undescribed structures are formed in the later stages of the mycelial inoculum culture. These originate from the 5 % of conidia which do not germinate during the usual period (four to twelve hours), but which continue to swell until by about the twenty-seventh hour they are  $10-12\mu$  in diameter. They are more frequent than, and therefore not identical with, the very large conidia in the original suspension. At about this time they germinate and produce one or two unbranched outgrowths,  $7-8\mu$  wide, with very densely staining contents and occasional septa. The ends of these outgrowths are frequently swollen and clavate in outline and are about  $10\mu$  wide. These forms contrast strikingly with the normal hyphae at this stage (Text-fig. 5 and Pl. XVIII, fig. 2). Withdrawal of the cytoplasm from the walls is more marked in these wide forms than in the normal hyphae. The little growth which occurs appears to be intercalary, not terminal, and is accompanied by a slight reduction in width.

During the later stages of the mycelial inoculum culture otherwise normal hyphae occasionally bear stunted swollen ends. These cannot be confused with the wide forms in which the contents are more homogeneous and stain more deeply.

#### HYPHAL DEVELOPMENT IN THE PENICILLIN FERMENTATION

Since the following observations are novel and relatively complex, a brief outline of the macroscopic changes occurring in the penicillin fermentation will first of all be given.

The mycelial inoculum grows vigorously until the entire volume of medium becomes dense with hyphae. The whole culture is then thick and moderately resistant to agitation. For convenience this may be described as the first 'generation' of hyphae. Within a few hours death of the greater portion of this 'generation' of hyphae results in the culture again becoming thin and mobile. A second 'generation' of mycelium arises from persistent elements of the first. Again a thick 'brei' is formed—the connotation is mash, porridge or pulp. Similar fluctuations, less pronounced in character, occur before the culture finally degenerates (Text-fig. 6).

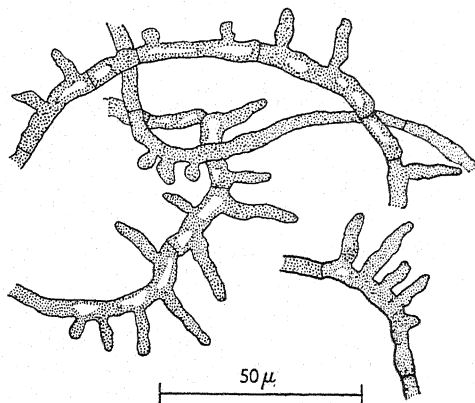


Text-fig. 6. The phases of growth occurring in the penicillin fermentation.

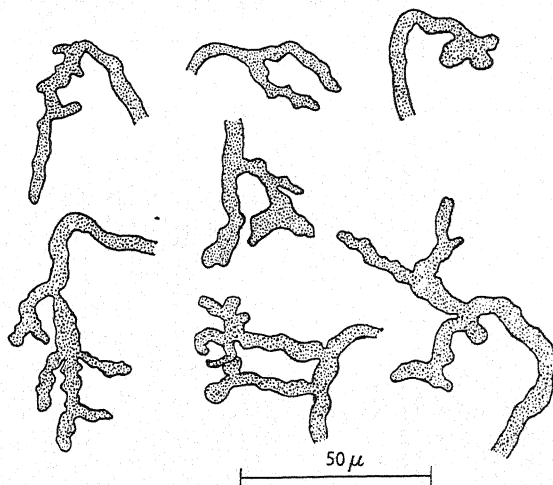
*First 'generation'.* When the mycelial inoculum is added to the penicillin-fermentation medium, numerous side branches rapidly develop on the existing hyphae (Text-fig. 7). These usually occur in groups very close together and may be  $30\ \mu$  long after three hours in the new medium (Pl. XVIII, fig. 3). After nine hours' stirring and agitation these side branches, some of which themselves possess branches, reach a length of  $250\ \mu$ . Their rate of growth is a little higher than that of newly germinated conidia in the mycelial inoculum culture.

Rapid growth of the normal hyphae continues for about sixty hours, during which time the hyphal tips become narrower and decrease gradually to a width of less than  $2\ \mu$ . The medium becomes filled with hyphae which produce a thick suspension or 'brei' (Pl. XIX, fig. 6). Before this occurs many of the hyphal tips show, for a short time, a peculiar distorted

appearance (Text-fig. 8). This effect is similar to that reported by Brian, Curtis and Hemming (1946) as being produced by the action of 'curling factor' on *Botrytis allii*, but in our cultures this appearance is transitory.



Text-fig. 7. The formation of numerous, grouped side-branches after three hours in the penicillin medium.

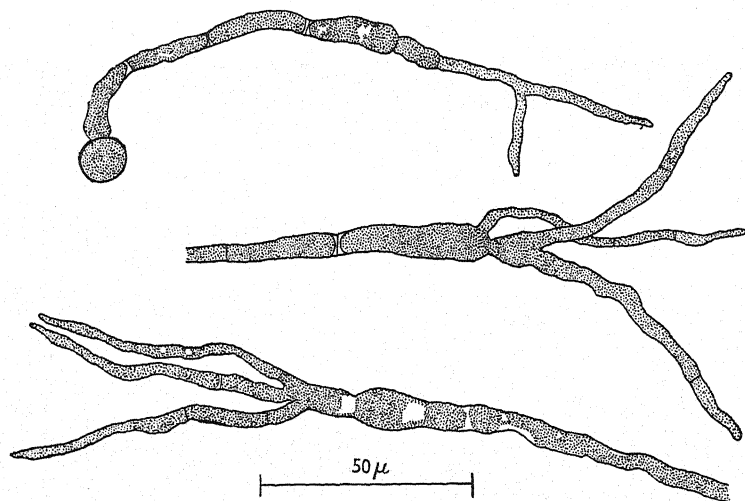


Text-fig. 8. Distorted stunted hyphal tips found at about the eighteenth hour in the penicillin fermentation.

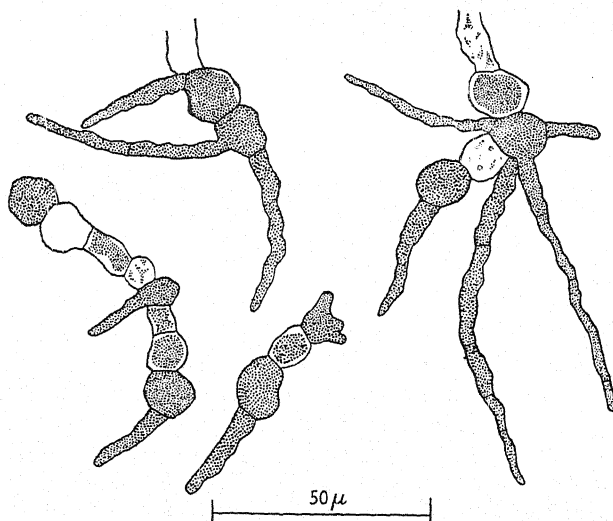
During this period empty hyphal walls are comparatively rare, but they rapidly become very common between the seventy-second and the seventy-eighth hour after inoculation. Since the walls themselves are not rigid the whole culture becomes thin and mobile.

The wide forms present in the mycelial inoculum develop in the penicillin-fermentation medium in some or all of the following ways: (a) further intercalary growth occurs so that at nine hours they may have reached 300 μ in length; (b) one or two densely staining non-vacuolated outgrowths

may be formed from the clavate end (Text-fig. 9): these are originally  $5-8\mu$  wide but later become indistinguishable from normal hyphae; (c) branches from the intercalary regions develop and produce hyphae which are normal in appearance; (d) large spherical cells are formed along the wide hyphae, singly or in short chains and usually in an intercalary



Text-fig. 9. Outgrowths from clavate tips in the early stages of the penicillin fermentation.



Text-fig. 10. Groups of large spherical cells producing densely staining outgrowths.

position. Their appearance is similar to that of the swollen conidia from which the wide hyphal forms originally arose. Some of these spherical cells become devoid of contents, but many retain their densely staining cytoplasm and form one or more outgrowths (Text-fig. 10 and Pl. XIX, fig. 5).

*Second 'generation'*. It is from these large spherical cells that the second 'generation' is largely produced (Pl. XIX, fig. 7). The outgrowths from them are at first 4-5  $\mu$  wide with densely staining contents and characteristically wavy walls, but as the first 'generation' dies off, they grow rapidly, branch and vacuolate. By ninety-six hours the macroscopic and microscopic appearance of the culture, apart from the presence of many empty walls, is very similar to that of the same culture at the fortieth hour (Pl. XIX, fig. 8). The hyphae of the second 'generation' become progressively narrower and more vacuolated so that by the hundred and eighth hour some, and by the hundred and thirty-second hour practically all, are empty. The culture then seems dead. However, even at this stage, another generation grows up and further, though less pronounced, fluctuations in the amount of living material follow, until, after the two-hundredth hour, the culture finally degenerates.

#### DISCUSSION

This communication is mainly descriptive and little attempt has been made to explain the phenomena observed; it is intended as an introduction to work on the isolation of specific factors responsible for the formation of the above-described characteristic structures. Some particular points are considered below.

A striking feature in these cultures is the diversity of mycelial form. The wide hyphae with clavate tips, the distorted endings and the large spherical cells are structures never previously associated with this species. In that they materially expand our knowledge of the morphology of the organism, the new forms here described raise a taxonomic problem, but it is perhaps better to suspend judgement on their significance.

It is possible that conidia which, by chance, germinate at a later stage in the inoculum media form abnormal hyphae as a result of some environmental condition produced by the growing mycelium. It is equally possible that these conidia are of a spontaneous variant which later reverts to the more vigorous parental type (Sartory, 1908; Sansome, 1947). Further work on this point is necessary.

The distorted endings occurring in the early stages of the penicillin fermentation are thought to be due to the vigorous nature of the agitation-aeration system. As the culture becomes more dense they rapidly disappear. These endings are similar in appearance to the 'degenerate reproductive structures' described and illustrated by Sartory in other species of *Penicillium* grown under conditions of agitation. However, penicilli, the elements of which are typical in size and dimensions though not in organization, do occur in the same cultures as distorted endings similar to those which we have described. It therefore appears probable that these distorted endings are the effect of environmental factors on vegetative hyphae and are not, as Sartory suggested, atypical penicilli.

The spherical cells differ from the 'forme de conservation résistante' observed by Sartory in that they possess no oil reserves and have walls which are only slightly thicker than normal. On morphological grounds they cannot be regarded as chlamydospores. On the other hand, in that



they tide the organism over periods during which conditions are unfavourable, they play the same physiological role.

The successive phases which occur in the penicillin fermentation show that it is not, as might perhaps be expected, a simple process involving the growth, maturation and senescence of the culture as a whole. The early rapid growth is followed by death of most of the hyphae, but persistent elements, the spherical cells which are directly descended from characteristic forms in the inoculum culture, form a new 'generation'. An explanation of these facts along the following lines was entertained.

As the amount of mycelium in the medium increases, the amount of oxygen available per living 'cell' falls, until a value is reached at which the oxygen requirements of the culture as a whole cannot be met. Presumably as a result of this, many of the 'cells' die, and in consequence a sudden large increase in the number of empty hyphal walls follows. This causes the culture to become thin and mobile. The oxygen supply to the residual living population is then adequate for renewed growth. The later cycles may be explained in the same way, though, in the impoverished medium, they will be less pronounced. In point of fact, data relating to oxygen transfer between air, medium and mycelium, subsequently obtained, support this explanation.

This description forms a basis upon which we hope to enlarge in future communications. It is hoped to examine the effect of environmental conditions on the morphology of agitated cultures of the *Penicillium notatum-chrysogenum* group. With such information available, it may be possible to define, by morphological examination, the conditions under which cultures have been grown and to correlate microscopic appearance with biochemical behaviour.

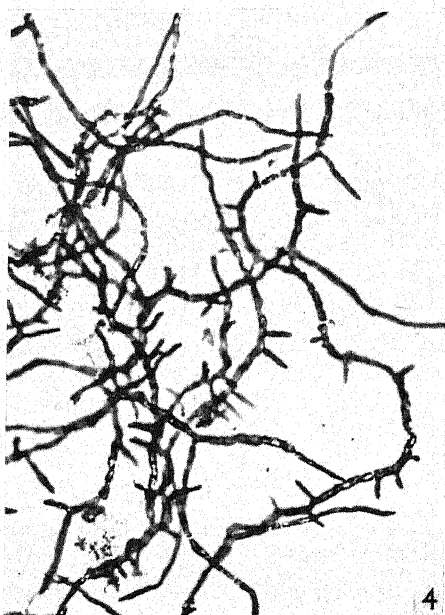
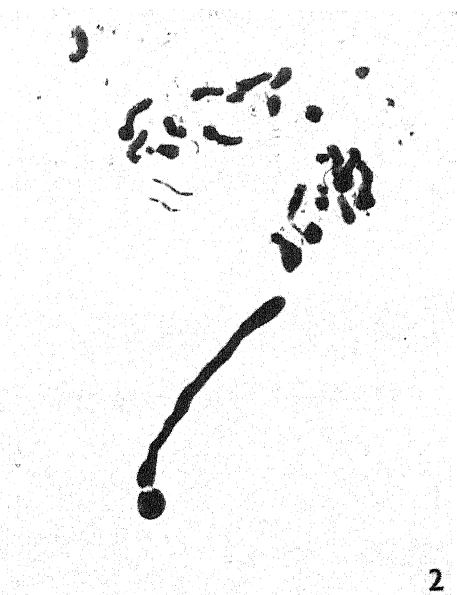
#### SUMMARY

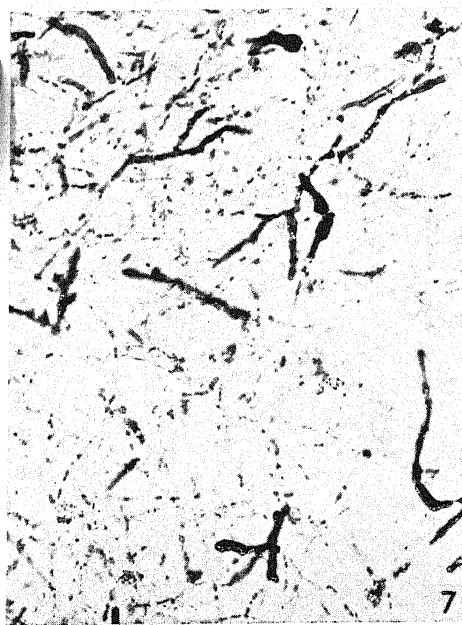
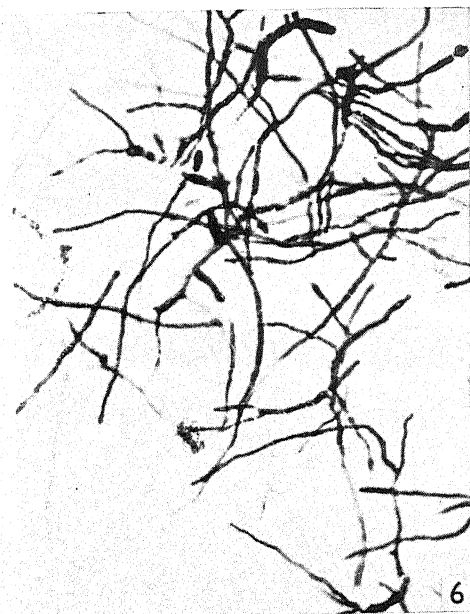
Conidia of *Penicillium chrysogenum*, Q 176 were inoculated into a liquid nutrient medium contained in a conical flask, which was then mechanically shaken for forty-eight hours (the mycelial inoculum culture). At this stage the mycelium so produced was transferred to a second liquid medium which was stirred and aerated in a bolthead flask (the penicillin fermentation). The morphology of these two cultures is described.

In the mycelial inoculum the majority of the conidia germinate between the fourth and twelfth hours to produce hyphae which branch and become progressively narrower. But some conidia (about 5 %) germinate later and form abnormally wide hyphae, usually with swollen tips.

The hyphae used to inoculate the bolthead flask rapidly produce, along their length, numerous side branches, growth of which soon fills the medium. Later these rapidly die. Meanwhile, the wide hyphae from the inoculum culture have formed spherical bodies which, at this stage, proliferate and form a second 'generation' of mycelium. Further fluctuations in the amount of living material follow until the culture finally degenerates.

The scope and aims of future work are indicated.





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# EXPLANATION OF PLATES

## PLATE XVIII

- Fig. 1. Hyphae from the inoculum medium at the fifteenth hour.  $\times 300$ .
- Fig. 2. A late-germinating swollen conidium at the thirtieth hour compared with conidia germinating during the normal period (four to twelve hours).  $\times 600$ .
- Fig. 3. Hyphae from the inoculum medium at the forty-eighth hour; a swollen spore with two outgrowths can be seen. Each outgrowth has a clavate densely staining tip.  $\times 300$ .
- Fig. 4. Hyphae from the penicillin fermentation after three hours; many short side-branches are present.  $\times 300$ .

## PLATE XIX

- Fig. 5. Large spherical cells from the penicillin fermentation at the forty-eighth hour.  $\times 1100$ .
- Fig. 6. Hyphae from the penicillin fermentation at the thirty-sixth hour.  $\times 300$ .
- Fig. 7. Hyphae from the penicillin fermentation at the seventy-eighth hour; many empty walls are present.  $\times 300$ .
- Fig. 8. Hyphae from the penicillin fermentation at the ninety-sixth hour; much new growth has taken place.  $\times 300$ .

(Accepted for publication 8 February 1948)

## STUDIES ON BRITISH CHYTRIDS

VII. ON *PHLYCTOCHYTRIUM MUCRONATUM* N.SP.

By HILDA M. CANTER

*The Freshwater Biological Association, Wray Castle, Ambleside and  
Department of Botany, Birkbeck College, University of London*

(With 2 Text-figures)

This chytrid was found growing as a saprophyte on *Closterium pritchardianum* Arch., inhabiting the mud surface, in the Clay Pond, Wray Castle, from late September to the end of November 1946 and in January 1947 on *C. costatum* Corda. in Blelham Bog, Wray Castle, Lancashire. At present it is included in the genus *Phlyctochytrium* as a new species, *P. mucronatum*, but when the nature of the resting spore, if any, is known this position may have to be revised.

The following description is based on observations of a large number of living specimens. All attempts to culture the chytrid on dead *Closterium*, on other algae, on pollen grains, and on *Daphnia*, failed. The zoospore settles on the host cell and puts forth a germ tube which penetrates the wall (Fig. 1e) and soon begins to branch. The rhizoidal system elongates, branches further and the portion immediately within the algal wall enlarges to form a small ( $1.3-3.5\mu$ ) spherical or subspherical apophysis. It seems that the apophysis is formed secondarily as a swelling of the germ tube after initiation of the rhizoids. Zoospores which had germinated in water under a cover-slip in the early stages of development showed no signs of an apophysis (Fig. 1d); however, they soon died and the subsequent formation of an apophysis could not be demonstrated. Two or three main rhizoidal axes may extend from the apophysis in large specimens, whereas small thalli may possess only one main branch. The extensive main axes often branch dichotomously, and taper towards their extremities.

The encysted zoospore is at first spherical with a single oil globule (Fig. 1e), it soon becomes more broadly ellipsoidal and often contains two oil globules (Fig. 1f). At first the apical spine is blunt (Fig. 1g), but as it elongates it becomes sharply pointed.

The subsequent changes in the protoplasm are similar to those found in most chytrids. It becomes finely granular, later small globules appear which coalesce to form the conspicuous oil globules of the zoospores. The mature sporangium exhibits a great variation in size and degree of ornamentation. An apical spine is always formed, and only once has a bifurcated specimen been observed (Fig. 1n). On the small sporangia  $8-13 \times 4-7\mu$  (Fig. 1i) no further spines develop, but as the sporangia become larger so the ornamentation increases. Some sporangia have, in addition to the apical spine, two oppositely placed median spines (Fig. 1j, m), others have from one to four whorls of spines. The very large specimens ( $31\mu$  in

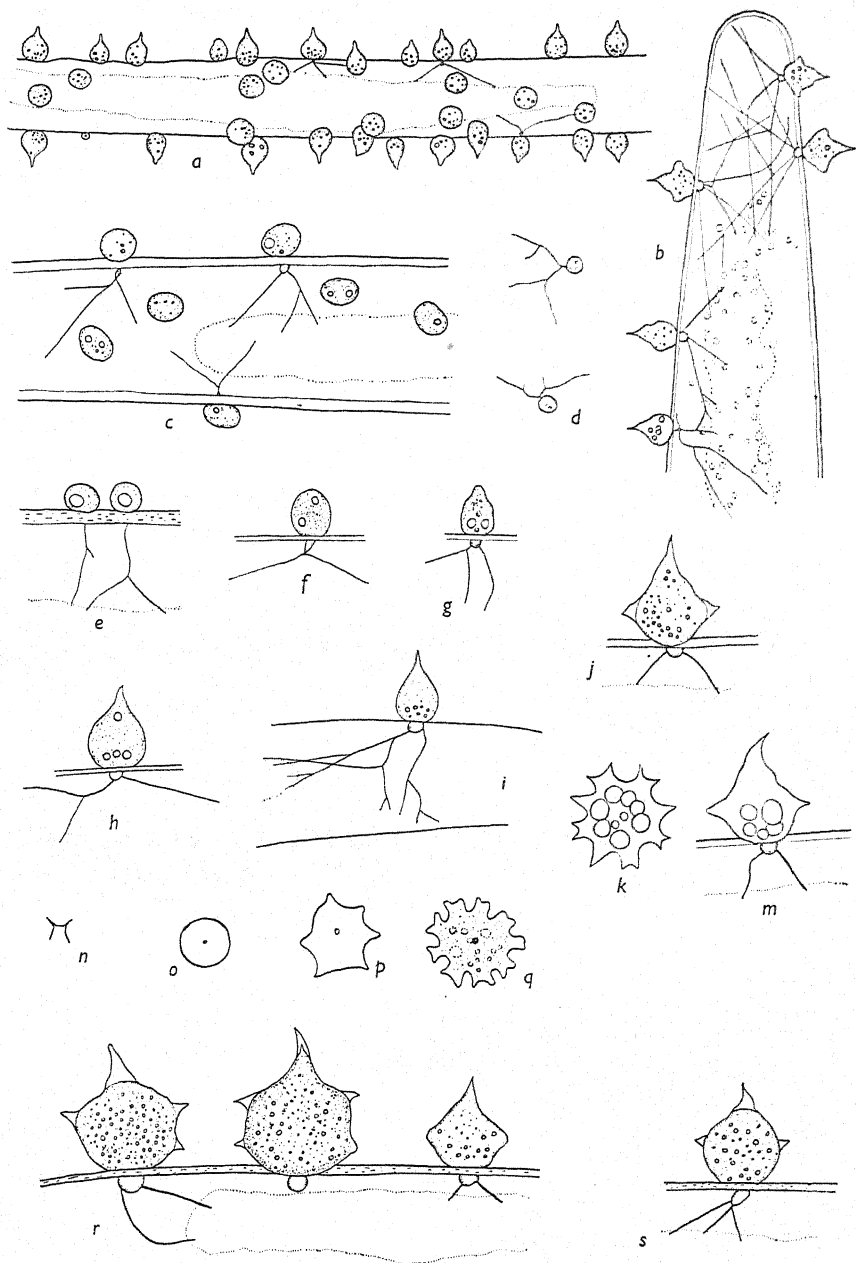


Fig. 1. *Phlyctocytrium mucronatum* n.sp. a, part of a *Closterium* cell with many young thalli. b, five larger sporangia, each with an apophysis and main rhizoidal axes. c, very young sporangia. d, early stage in germination of two zoospores under a cover-slip. e-i, stages in development of thalli with only an apical spine. j, immature thallus with two lateral spines. k, m, thallus with single median spine whorl; some spines in the whorl forked, others simple. n, forked apical spine. o-q, sporangia viewed from above. o, no median spines; p, simple spines; q, forked spines. r, s, larger thalli with one and two spine whorls. a, b, d,  $\times 450$ ; h,  $\times 720$ ; c, e-g, i-s,  $\times 945$ .

diameter including the spines) with four spine whorls (Fig. 2c) are rare and usually the basal whorl is less well developed than the rest. The spines may be bifurcated or simple and both types may occur in the same whorl (Fig. 1k); only their extreme apices are solid. The size of the sporangium shows no correlation with the number of parasites occurring on the host cell. Small sporangia often occur in large numbers (up to 100) on a host

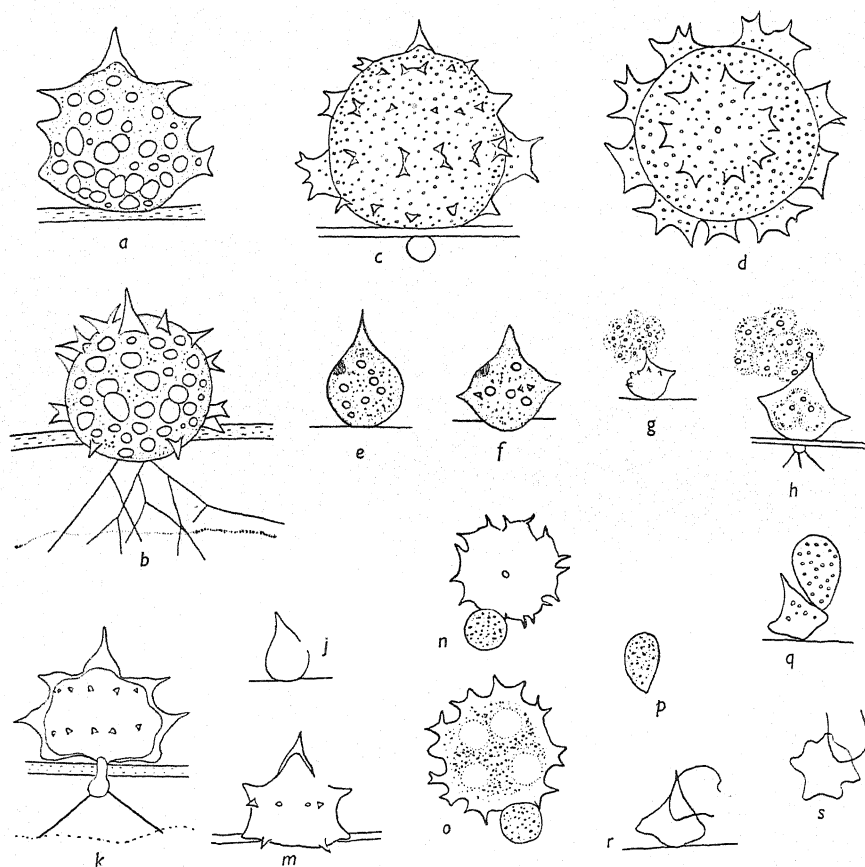


Fig. 2. *Phlyctochytrium mucronatum* n.sp. a, sporangium with three spine whorls. b, the same as (a) showing main rhizoidal axes. c, four-spine whorled thallus. d, (c) viewed from above. e, f, mature thalli with lateral dully refractive area marking the place of dehiscence. g, h, sporangia dehiscing. i, j, k, m, empty sporangia. n-p, hyperparasite in different views on the same sporangium. q, mature sporangium. r, s, empty sporangia. a-f, h-m,  $\times 945$ ; n-p, r, s,  $\times 765$ ; g,  $\times 450$ ; q,  $\times 720$ .

cell, but there is no indication that they are impoverished specimens due to overcrowding. Large sporangia with two to four lateral whorls of spines develop beside them and isolated, small sporangia are common. When mature, the sporangium contains a number of spherical refractive globules each of which indicates the position of a zoospore. The number of zoospores formed in a sporangium varies according to its size; three to eight in small



sporangia, thirty to sixty in large ones. In a few mature sporangia a dully refractive area (Fig. 2e, f) was seen just below the apical spine, which probably marked the region of dehiscence. On dehiscence of the larger sporangia, part of the sporangial wall between the apical spine and first whorl of spines deliquesces to form a pore. In the smaller sporangia this dehiscence pore is more median in position (Fig. 2j). A dehiscence papilla is never developed. The contents of the sporangium emerge to form a mass outside; although no definite vesicle was observed continuous with the sporangium, the zoospore mass is held together by some invisible substance. If this mass is moved while under a cover-slip, it remains connected with the sporangium. After a minute or so the individual zoospores are delimited, although still entangled by their flagella. They soon become freed from one another and swim away individually with a rapid darting movement. The zoospores are spherical,  $4-5\mu$  in diameter, with a conspicuous posterior oil globule, above which is a clearer area containing a refractive granule; the remainder of the protoplasm is coarsely granular (Fig. 2h).

In spite of the great range of thallus structure it seems that only one species is concerned, since intermediate stages from small thalli with only an apical spine to the thalli with four spine whorls are to be found. A few specimens of this chytrid were parasitized by an unidentified species of the same order (Fig. 2n-p). Although the sporangia resemble *Septosperma anomalum* (Couch) Whiffen (which has recently been found by me on *Chytrium tabellariae* (Schröter) Canter in a nearby bog), it cannot be definitely assigned to this species until the resting spore is discovered.

*Phlyctochytrium mucronatum* more especially resembles the dentigerate members of this genus (see Sparrow, 1943, pp. 229-34), e.g.: *P. planicorne* Atkinson, *P. zygnetatis* (Rosen) Schroeter, *P. quadricorne* (de Bary) Schroeter, *P. bullatum* Sparrow, *P. urceolare* Sparrow, *P. dentiferum* Sparrow and *P. aureliae* Ajello (1945). They have all been observed as saprophytes; in the first three and *P. aureliae* the zoospores are described as emerging in a mass from the sporangium and in none is the resting spore known. However, the wide variation in thallus structure exhibited by *P. mucronatum* has not been recorded for the other species. The large sporangia most closely resemble those of *P. aureliae* except that in the latter the teeth are scattered over the surface in an apparently haphazard fashion and by proliferation of their apices may become setigerous or thread-like. Again in *P. aureliae* the zoospores emerge by way of a rupture in the sporangial wall as in *P. mucronatum*. In other *Phlyctochytrium* spp. an apical dehiscence papilla, surrounded by a collarette of teeth is formed. The characteristic apical spine clearly distinguishes *P. mucronatum* from *P. aureliae*. In its saprophytic habit, apophysis, extensive rhizoidal system and emergence of the zoospores in a mass, *P. mucronatum* shows a superficial resemblance to the exuviate chytrids, especially the genus *Asterophlyctis*. As mentioned earlier (p. 236), until the resting spores are known the exact affinities of this organism remain obscure, but there is little doubt that the chytrid here described represents a new species. The following diagnosis is suggested.

**Phlyctochytrium mucronatum** sp. nov.

Thallus epibioticus, monocentricus, e sporangio, apophysi et rhizoideis extensis compositus. Sporangia subglobosa,  $5.7-31\ \mu$  diam. (spinulis inclusis) spinulam singulam pyramidatam apicalem  $1.4-5.2\ \mu$  longam, basi  $4.3\ \mu$  latam, et spinulas laterales (in sporangiiis parvis deficientes) simplices vel furcatas in verticillis 1-4 dispositas gerentia, poro laterali dehiscencia, cytoplasma integra emergente. Zoosporae sphaericae  $4-5\ \mu$  diam., intus crasse granulosae, postice globula oleosa et supra spatio clariore et granulo nigro praeditae. Apophysis sphaerica,  $1.3-3.5\ \mu$  diam. Rhizoidea ramosa, axibus principalibus 1-3 ex apophysi oriundis, saepe dichotome ramosis, apices versus angustatis, ad  $85\ \mu$  longis. Sporae perdurantes non visae.

Hab. Saprophyticus, in *Closterio pritchardiano* Arch. et *C. costato* Corda, Clay Pond et Blelham Bog, Wray Castle, Anglia, 1946.

**Phlyctochytrium mucronatum** n.sp.

Thallus epibiotic, monocentric, consisting of a sporangium, apophysis and extensive rhizoidal system. Sporangia more or less spherical  $5.7-31\ \mu$  in diameter (including spines), with a single pyramidal apical spine  $1.4-5.2\ \mu$  long  $\times$   $0.9-4.3\ \mu$  broad at the base and with one to four whorls of lateral simple, or Y-shaped spines (absent in very small sporangia). Sporangium dehiscing by a lateral pore; contents emerging in an undifferentiated mass continuous with the sporangium. Zoospores spherical,  $4-5\ \mu$  in diameter, with a posterior oil globule, above which is a clearer area with a black granule; remainder of the protoplasm is coarsely granular. Apophysis spherical  $1.3-3.5\ \mu$  in diameter. Rhizoidal system one to three main axes leaving the apophysis, often branching dichotomously and tapering towards their extremities, up to  $85\ \mu$  long. Resting spores not observed.

Saprophytic on *Closterium pritchardianum* Arch. and *C. costatum* Corda, the Clay Pond, and Blelham Bog, Wray Castle, England, 1946.

My thanks are due to Miss E. M. Wakefield of Kew for the Latin translation and to Prof. C. T. Ingold for reading the manuscript.

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## LEAF DISEASES OF *ANEMONE CORONARIA* IN CORNWALL

By P. H. GREGORY, *Rothamsted Experimental Station,  
Harpenden, Herts.*

(With Plate XX and 2 Text-figures)

In the ten years before 1940 the winter production in the open air of cut flowers of *Anemone coronaria* increased rapidly in south-west England. The crop is grown either from seed sown in March or April, or from 1-3 cm. corms, usually of Dutch origin, planted in July (Johnstone & Walbank, 1938). The notes which follow describe the leaf diseases of anemone observed during the years 1935-40.

**WINTER BROWNING.** The most important anemone disease in the south-west has been named Winter Browning by Beaumont and Staniland (1935), who described the course of the disease and discussed possible causes. It usually appears in the winter months and may be associated with root decay, a purplish tint to the leaf, and decay of the leaf margin. The corm is normally unaffected, but the flower crop is seriously reduced, and flowers already formed are small and brown at the edges. The disease spreads to form large patches which may eventually cover the entire field. In moist dull weather diseased tissues may become covered with a copious growth of *Botrytis cinerea*, but inoculations with strains isolated from diseased plants have failed to reproduce the symptoms on healthy plants. The cause of winter browning is not yet known, but the possibility of it being caused by a virus, such as spotted wilt or cucumber mosaic virus, needs to be investigated. Anemones grown from seed usually remain free from the disease, while it often devastates fields planted with corms.

**CLUSTER CUP RUST** (*Puccinia pruni-spinosae* Pers.) rarely occurs in commercial plantings of *Anemone coronaria*, which is grown as an annual crop. When beds are left for a second year, however, plants bearing hypertrophied leaves with pycnidia and aecidia of the rust often develop in spring. *A. fulgens* grown as a perennial from corms imported from France may show up to 50 % of diseased plants. Affected shoots flower poorly, if at all. A corm may be only partly infected and produce both normal and hypertrophied shoots. Domestic and wild plum are commonly attacked in the south-west.

**POWDERY MILDEW** (*Oidium* sp.) was first recorded at Penzance on 17 October 1935 on anemones grown from corms. The attack was slight and the fungus appeared as a thin white, powdery layer in small patches on otherwise normal leaves, petioles and flower stalks. Microscopic examination showed numerous superficial radiating mycelia on apparently healthy parts of the epidermis, producing abundant spores of *Oidium*. The perithecial stage has not yet been recorded on *Anemone coronaria* and the identity of the fungus must remain in doubt. Beaumont (1938) has indicated that it is probably *Erysiphe polygoni* DC. Traces of the mildew

were found each autumn, but it became severe only in the middle of October 1937 when six acres of St Brigid and anemone de Caen at St Erth, Cornwall were badly affected. The plants, grown from corms from various sources on a north-west and west slope and obviously suffering from the effects of drought, were so heavily infected with the mildew that the field looked as though it had been lightly dusted with lime. The characteristic aromatic odour of powdery mildew was obvious. The conidiophores were most numerous on the laminae of the older leaves, but were also present on the younger leaves. The flowers were poor with short stems. At the same date the disease was severe on anemone de Caen grown from seed at St Hilary. In September this field was yellow from drought, but by the middle of October it had recovered and appeared a healthy green except where whitened by the mildew. By December new leaves had been produced which remained free from attack, and the fungus could be found only after a search on the older leaves.

DOWNY MILDEW (*Plasmopara pygmaea* (Unger) Schroet.) was first reported on *Anemone coronaria* in Cornwall by Beaumont (1938). It was observed at the end of September 1937 on a field of anemone de Caen near Liskeard, where the foliage of a patch three-quarters of an acre in extent became blackened and died in about four days. Conidiophores of a downy mildew were apparent with a hand-lens, maximum conidial production being from stomata on portions of the green leaf immediately outside the blackened zone. The fungus was readily identified as *Plasmopara pygmaea* (Text-fig. 1). Oospores were formed in the discoloured tissues of the leaf. By the beginning of December the crop had recovered, no *Plasmopara* could be found and the plants were producing flowers again.

DOWNY MILDEW (*Peronospora ficariae* Tul.). A second downy mildew on *Anemone coronaria* and one which is commoner than *Plasmopara pygmaea* though usually much less conspicuous, may be referred provisionally to *Peronospora ficariae* Tul. Its occurrence has been reported by Moore (1943), and it is apparently the first *Peronospora* to be found on any species of anemone. It was first noticed in September 1935 during microscopic examination of apparently healthy leaves of anemone de Caen collected at Penzance. Many leaves were free from the fungus, but on others isolated sporangiophores could be found protruding through a few of the stomata. Often a large area of leaf had to be searched before a single specimen could be found. These sparsely distributed sporangiophores were never, in this collection, associated with visible lesions, and the fungus could not be seen with the naked eye. Further collections from the same field in the next few weeks yielded some more material, but it disappeared during the winter. It was not possible to ascertain whether infected leaves subsequently died. Early in December 1936 the disease appeared in a more severe form in a garden at Newton Abbot where four beds of *Anemone coronaria* were blackened. The leaves had blackish necrotic marginal lesions and were covered with conidiophores of the downy mildew visible to the naked eye or with a hand-lens. The affected leaves were rolled upwards, thus bringing most of the sporangiophores, which protruded through the stomata on the lower surface of the leaf, into an erect position (Pl. XX, fig. 1). The bracts were also affected. Sporangia germinated by zoospores,

a character associated with *Plasmopara*, rather than *Peronospora*, but germination by zoospores has been reported in *P. spinaciae* by Eriksson (1918). The conidiophores (Text-fig. 2), however, were entirely different from *Plasmopara* and resembled a figure of *Peronospora ranunculi* Gäumann (1923), a species which is also a member of what Gäumann considers to be the *Formenkreis* *P. ficariae* Tul. The sporangia became swollen in water and before germination measured  $28-39 \times 23-30 \mu$ . Oospores were not seen in any of the material examined. It is possible that *P. ficariae* is more wide-

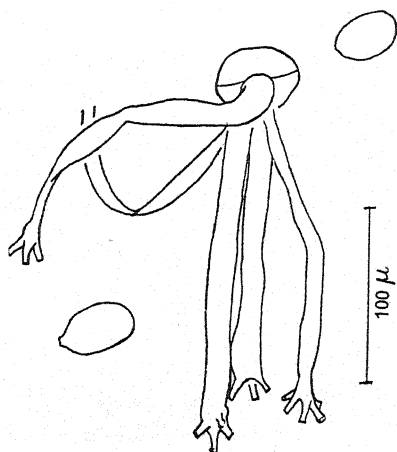


Fig. 1

Text-fig. 1. *Plasmopara pygmaea* (Unger) Schroet. Camera lucida drawing of conidiophores and conidia from leaf of *Anemone coronaria*, Liskeard, Cornwall, 27 September 1937.

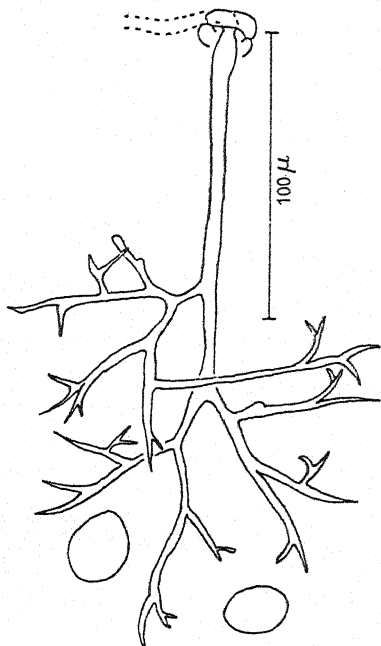


Fig. 2

Text-fig. 2. *Peronospora ficariae* Tul. Camera lucida drawing of conidiophores and conidia on leaf of *Anemone coronaria*, Penzance, 11 October 1935.

spread on anemones and does more damage than these records suggest. Further investigation may show how often blackening of anemone foliage is the sequel to production of a crop of conidia and disappearance of the conidiophores.

**BLACK LEAF SPOT** (*Septoria anemones* Desm. var. *coronariae* Grove). This disease occurs occasionally in autumn, forming conspicuous dark brown or black spots on mature leaves, usually near the margins (Pl. XX, fig. 2). The diseased tissue is dry and sunken and deeply discoloured with a brownish black pigment. The blackened area is sharply demarcated from the surrounding healthy green area. Examination of the blackened area with a hand-lens shows the presence of numerous black pycnidia from which masses of spores are exuded in cirri.

The disease was first noted on a small plot of anemones at Gulval, near Penzance, Cornwall, on 16 September 1936. The spots were particularly numerous where the plants were shaded by a *Pittosporum* hedge. The ground had previously carried a crop of lettuce and had subsequently received a dressing of farmyard manure and fish manure, before being planted with 1-2 cm. anemone corms at the end of May. Several varieties were affected including de Caen, St Brigid and Hollandia. The disease spread over the plot during October and November and persisted through the winter. During March 1937 the foliage on the affected beds was killed by strong dry winds and the new foliage which grew later remained free from the disease. The disease was not observed on the 1937-8 crop, but reappeared within a few hundred yards of the first outbreak in December 1938 on anemones grown from seed sown the previous March.

*Septoria anemones* Desm., which is said to be common on our wild *Anemone nemorosa*, was described by Grove (1935-7) as having 'spots dry, at length brown... not bordered, sometimes hardly different in colour from the rest of the leaf, greenish or brown, paler in the centre'. The spores measure  $20-22 \times 1-1.25 \mu$ .

Microscopically the fungus on *A. coronaria* resembles *Septoria anemones* Desm. except that the spores are longer, measuring  $30-42 \mu$  in the Cornish material. The late W. B. Grove, to whom specimens were submitted, stated that the length of the spores was not sufficient to differentiate the specimens on *Anemone coronaria*, but he considered that the black colour of the spots justified making a new variety. He accordingly described *Septoria anemones* var. *coronariae* as producing 'spots dark-brown or almost black, large, well-defined, mostly marginal, irregular in form, but not bordered with another colour. On leaves of *Anemone coronaria*, Penzance (Gregory). Oct.' (Grove, 1935-7). The leaves of *A. coronaria* readily turn dark brown on wounding or death from any other cause, and it is possible that the colour of the spots is in part a character of the host.

The fungus grows readily and produces spores on artificial media (glucose-peptone and glucose-asparagin agar).

Specimens and slides of the *Oidium* sp., *Plasmopara pygmaea*, *Peronospora ficariae* and co-type material of *Septoria anemones* var. *coronariae* on *Anemone coronaria* have been deposited in the Herbarium of the Ministry of Agriculture, Plant Pathology Laboratory, Harpenden.

I carried out this work under a scheme financed jointly by the Great Western Railway Co. through the Cornwall Farmers' Union and the Ministry of Agriculture and Fisheries, upon the recommendation of the Agricultural Research Council, to whom grateful acknowledgements are made. Thanks are also due to Mr A. Beaumont and Mr W. C. Moore for help given in the course of the work.

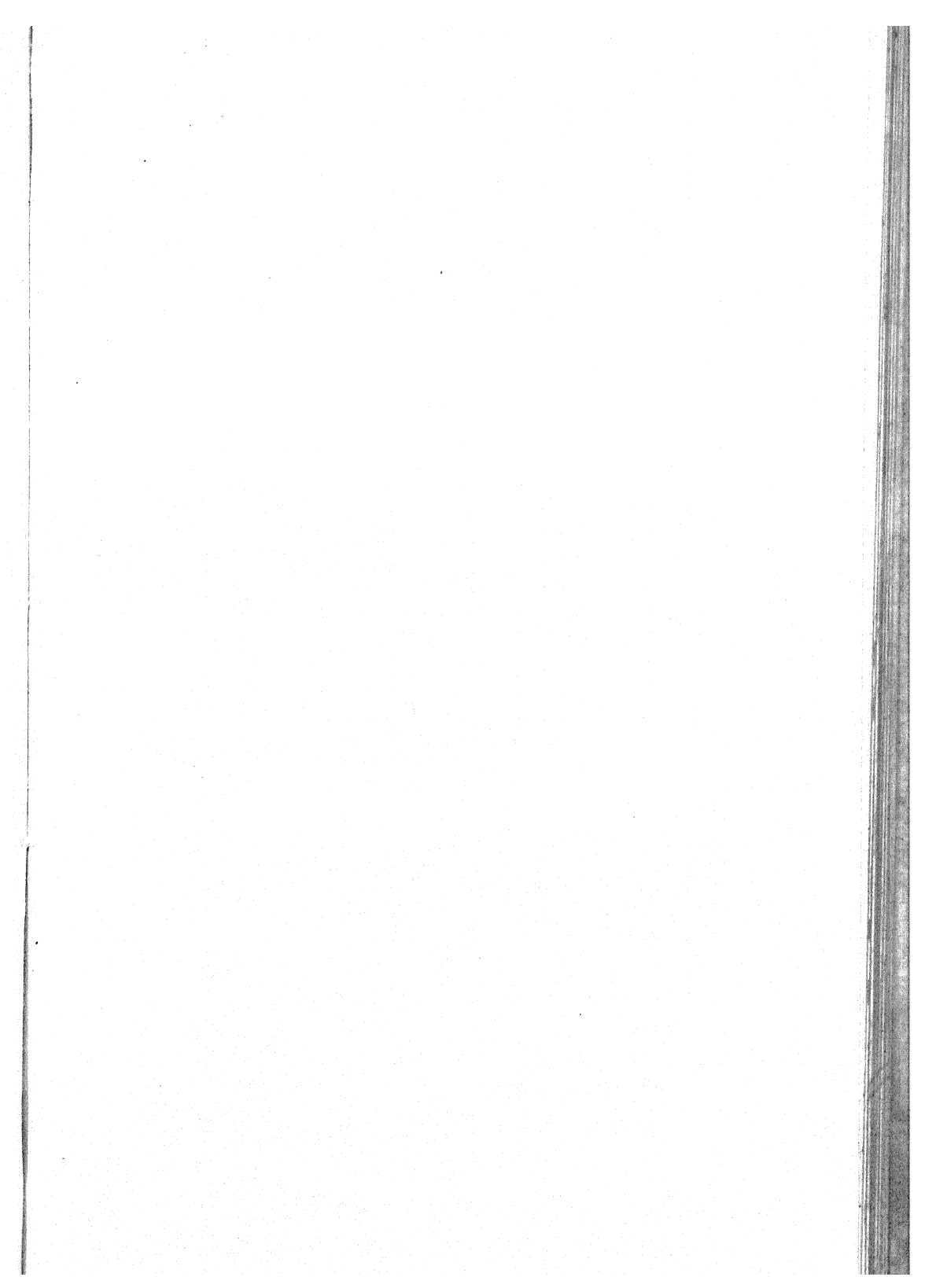






Fig. 1

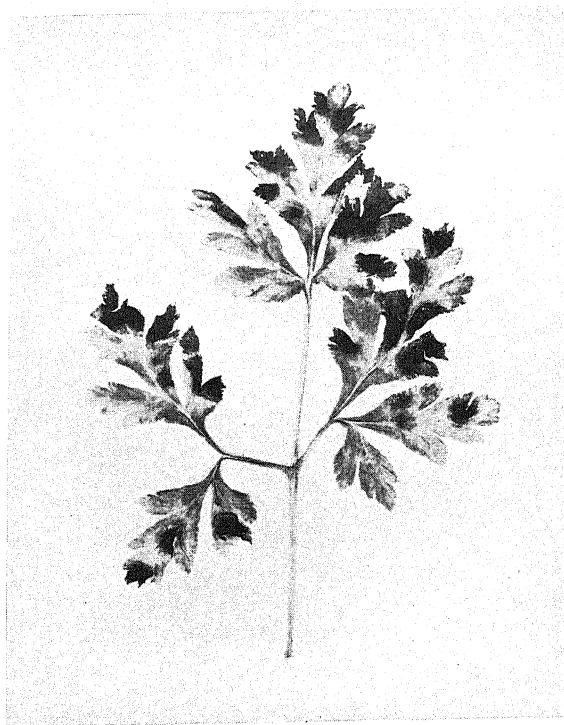


Fig. 2.

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# EXPLANATION OF PLATE XX

- Fig. 1. Leaf of *Anemone coronaria* attacked by *Peronospora ficariae* Tul., Newton Abbot, December 1936.
- Fig. 2. Leaf of *Anemone coronaria* attacked by *Septoria anemones* var. *coronariae* Grove, Penzance, 16 September 1936.

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## TETRAPLOA

By M. B. ELLIS, *Commonwealth Mycological Institute, Kew*

(With 2 Text-figures)

Up to the present time five species of *Tetraploa* have been described: *T. aristata* Berk. & Br. (the type species), *T. divergens* Tracy & Earle, *T. ellisii* Cooke, *T. muscicola* Gonz. Frag., and *T. scabra* Harkn. Only brief descriptions of these fungi have been published. In this paper *T. aristata* and *T. ellisii* are redescribed and an account is given, for the first time, of cultural characters and the development of the spores in the former; notes on the other species are appended.

I. *TETRAPLOA ARISTATA* Berk. & Br.

The original description of *T. aristata*, drawn up by Berkeley and Broome (1850), was based on material on grass collected at Westhay Woods, King's Cliffe, Northants. Since 1850, surprisingly few records of this easily recognized fungus have been made, in spite of the fact that it occurs throughout the year on a number of grasses, sedges and other plants, and has a wide geographical distribution. In Great Britain it has been recorded on *Phalaris arundinacea* (Stevenson & Trail, 1885), on an unnamed host (Bucknall, 1886), on grass, probably *Poa pratensis*, cut and thrown into a heap, and on living roots of grass (Bayliss Elliott & Stansfield, 1923), on *Cladium mariscus* (Ellis, 1941 and B.M.S. Foray Report, 1947), on *Phragmites communis* (Ellis, 1941), on *Angelica sylvestris*, *Heracleum sphondylium* and *Pteridium aquilinum* (E. A. Ellis in litt.). Thirty collections were made in this country between 1940 and 1947 on dead leaves and stems of *Ammophila arenaria*, *Carex paniculata*, *Cladium mariscus*, *Cortaderia selloana*, *Cyperus longus*, *Phragmites communis* and wheat stubble. The spring gatherings showed large numbers of young developing spores; in the late summer, autumn and winter gatherings, mature spores predominated. Outside Great Britain the fungus has been recorded on *Ammophila arenaria* in Holland and on *Avena pratensis* [*Helicotrichon pratense*] in Germany (Lindau, 1908), on *Bambusa* in China (Wei & Hwang, 1924-37), on *Festuca* in Italy (Ferraris, 1912), on *Gynerium argenteum* [*Cortaderia selloana*] in Eire (Pim, 1884), on *Saccharum officinarum* in Alabama, Barbados, Cuba, the Dominican Republic and Puerto Rico (Stevenson & Rands, 1938), on straw in Denmark (Lind, 1913), on wheat stubble in Holland (Van de Laar, 1931) and on an unnamed host in Japan (Shirai & Hara, 1927). Two recent gatherings from Africa have been examined, one on *Anadelphia leptocoma* collected in Sierra Leone by F. C. Deighton, and the other on *Cymbopogon afronardus* collected in Uganda by C. G. Hansford.

Colonies of the fungus on the host are brown or dark greyish brown in colour; they are either very small and localized, or else they form an irregular mat spreading right round the leaf or stem and four to five

centimetres along it. The mycelium is superficial and is made up of a web of branched and anastomosing septate hyphae  $1.5-3\ \mu$  in diameter, hyaline to pale yellowish brown, with smooth to verruculose walls (Fig. 1 S).

Two kinds of conidia are formed directly on the mycelium: (a) large ones with setose appendages about the length of the spore (Fig. 1 Q, R), and (b) small conidia with very long appendages (Fig. 1 T, U). Probably the larger conidia only were observed by Berkeley and Broome who likened them aptly to 'the achaenium of some composite plant'. Each conidium arises as a small bud with deeply staining cell contents (Fig. 1 A-D and H); this bud swells and becomes divided into two by a vertical wall (Fig. 1 E, I); each of the two cells thus formed is divided again by a second vertical wall at right angles to the first (Fig. 1 F, J). No further walls are laid down in a vertical plane, but each of the four initial cells swells, elongates, and as a rule becomes divided by horizontal walls into a column of from two to six cells (Fig. 1 G, K and L). In mature spores there are shallow furrows between the columns of cells which are formed independently and tend to diverge from one another apically. The columns are sometimes of unequal length; in each, the terminal cell puts out a process (Fig. 1 M-O) which elongates and becomes a septate setose appendage. Cotton blue in lactic acid stains the rounded contents of each cell and shows up strands of protoplasm linking them together. There is apparently protoplasmic continuity by pores between the four initial cells, the individual cells in each column, the terminal cells and the setose appendages (Fig. 1 R). Mature conidia are brown, thick-walled and verrucose; the appendages are smooth, brown at the base and almost hyaline at the tips. Measurements and structural details of the two kinds of conidia are tabulated below:

	Average of twenty conidia ( $\mu$ )	
	(a)	(b)
Body of conidium:		
Length	25-39 (31.8)	8-18 (12)
Diameter at base	7-14 (10.7)	7-12 (9.5)
Maximum diameter	14-29 (20.6)	9-16 (12.3)
Number of cells in each column	3-6	1-2
Setose appendages:		
Length	12-80 (36)	80-330 (218)
Diameter at base	4.5-8 (5.97)	3-6 (4.97)
Diameter at apex	2-3.5 (2.45)	1-2 (1.97)
Number of septa	1-6	3-12

*Tetraploa aristata* is easily grown on potato-dextrose agar. Germ tubes are produced from the body of the conidium and from the ends of the setae. Spores germinated on 27 August 1947 and grown at room temperature formed by 8 September greyish brown, freely sporing, colonies 12-14 mm. in diameter; the conidia formed did not differ in any way from those produced on natural substrata. The septate, branched mycelium, composed of hyphae  $2-3\ \mu$  in diameter, appeared pale yellowish brown by transmitted light and was seen to be verruculose.

The spores of this fungus with their long appendages resemble those of certain aquatic hyphomycetes and it is thought that water aids in their

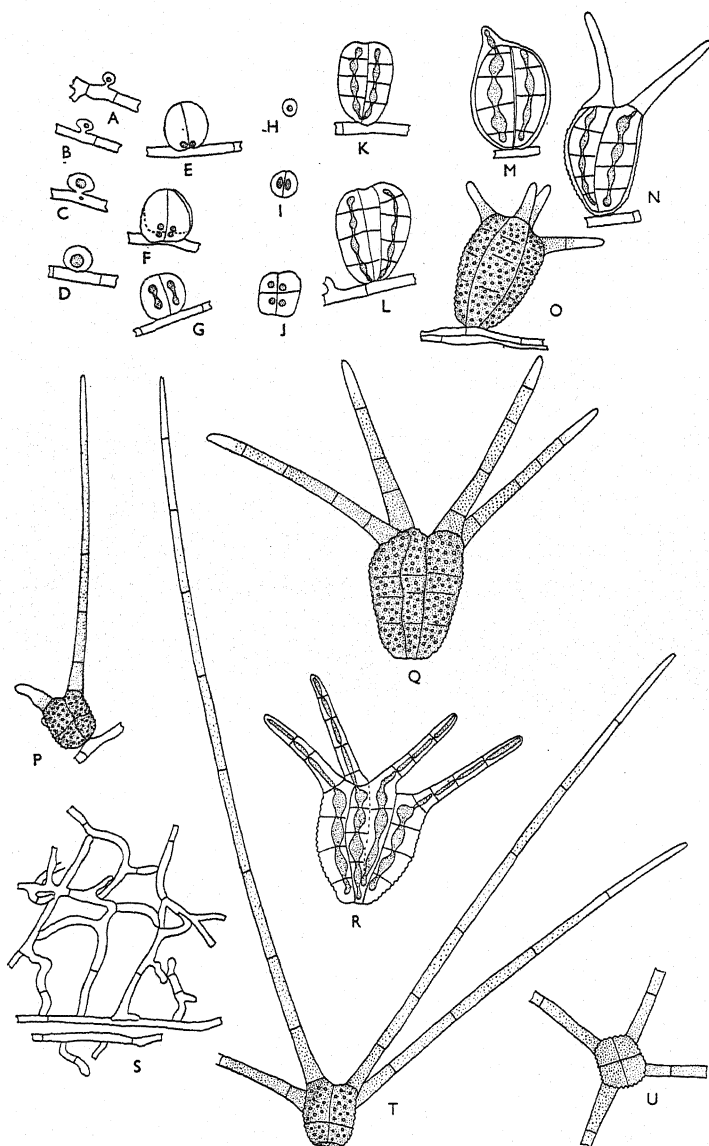


Fig. 1. *Tetraploa aristata*. A-O, development of a spores; P, young b spore. Q, mature a spore; R, a spore stained to show protoplasmic linkage by pores; S, mycelium; T, lateral view of b spore; U, end view of b spore. (All  $\times 500$ .)

dispersal. When an infected leaf or stem is covered with a thin film of water a number of spores break off and float to the surface; on tilting they move down with the flow. Spores mechanically detached from the substratum and placed in a 250 c.c. beaker full of water floated for several hours; when submerged they sank at the rate of 1.5 cm. per minute.

*T. aristata* is usually found on leaf bases and on stems just above the soil although it also grows at a much higher level on dead standing leaves of *Cladium* at Wheatfen Broad. Most of the recent collections of this fungus have been made on marshes and fens subjected to periodic flooding and here it is probable that flood water plays at least some part in spore dispersal. Spores of a number of marsh fungi, including *Tetraploa aristata*, have been found floating on the surface of dykes.

Specimens examined:

On *Ammophila arenaria*, Scratby, Norfolk, 26 August 1945, E. A. Ellis (Herb. I.M.I. No. 23223).

On *Anadelphia leptocora*, Kenema, Sierra Leone, 6 July 1937, F. C. Deighton (Herb. I.M.I. No. 22000).

On *Carex paniculata*, Wheatfen Broad, Norfolk, 27 December 1946, E. A. Ellis (Herb. I.M.I. No. 10523).

On *Cladium mariscus*, Wheatfen Broad, Norfolk, 1940-7, E. A. and M. B. Ellis: January (Herb. I.M.I. No. 10323), March (Herb. I.M.I. Nos. 21397 and 21398a), April (Herb. I.M.I. Nos. 14875h, 16534b, 21400 and 21401a), May (Herb. I.M.I. Nos. 8998a, 15331, 15393c and 15413f), June (Herb. I.M.I. Nos. 8999 and 21440), August (Herb. I.M.I. Nos. 16670 and 17342), September (Herb. I.M.I. No. 21441), November (Herb. I.M.I. Nos. 8911a, 8912 and 21442), December (Herb. I.M.I. Nos. 10888, 21149c and 21276b); Derby Fen, Grimston, West Norfolk, 26 August 1945, E. A. Ellis.

On *Cortaderia selloana*, Perranzabuloe, West Cornwall, 27 May 1947, F. Rilstone (Herb. I.M.I. No. 15540a).

On *Cymbopogon afronardus*, Kisube, Entebbe, Uganda, October 1944, C. G. Hansford (Herb. I.M.I. No. 178).

On *Cyperus longus*, Grand Mare, Guernsey, Channel Islands, 6 July 1939, E. A. Ellis (Herb. I.M.I. No. 21444).

[On grass], Westhay [Woods, King's Cliffe, Northants]; ex Herb. Berk. 1879, in Herb. R.B.G. Kew (slide filed as Herb. I.M.I. No. 17841). Type.

[On grass], labelled *Tetraploa aristata* in C. E. Broome's handwriting; ex Herb. R.B.G. Kew (slide filed as Herb. I.M.I. No. 17842).

On *Phragmites communis*, Wheatfen Broad, Norfolk, April, E. A. and M. B. Ellis (Herb. I.M.I. Nos. 14514c and 21443).

On wheat stubble, Newton St Cyres, Devonshire, 18 September 1947, M. B. Ellis (Herb. I.M.I. No. 17534).

## 2. *TETRAPLOA ELLISII* COOKE

The first description of *T. ellisii* Cooke, published by Cooke and Ellis (1879), was based on material on old stalks of *Zea mays* collected at Newfield, New Jersey, in 1878. The fungus appears to have been recorded only from North America where it occurs on the type host and on *Arundinaria tecta*. The colonies are brown and cover an area of 3 sq.cm. or more. The superficial mycelium is composed of hyaline to pale brown, branched septate hyphae, 2.5-4  $\mu$  in diameter, with verruculose walls. Only one type of conidium has been seen in which the appendages are on the whole longer than those of the *a* spores and shorter than those of the *b* spores of

*Tetraploa aristata* (Fig. 2). The body of the spore is brown, smooth or verrucose (often verrucose only at the base) and made up of three or four columns of cells each of which ends in a gradually attenuated, smooth-walled, septate appendage. Measurements and structural details of the conidia are given below:

	Average of ten conidia ( $\mu$ )
Body of conidium:	
Length	30-51 (39)
Diameter at base	10-18 (13)
Maximum diameter	15-26 (20.6)
Number of cells in each column	4-8
Setose appendages:	
Length	24-178 (97)
Diameter at base	7-10 (8.7)
Diameter at apex	2-4 (2.8)
Number of septa	2-18

Specimen examined:

On old stalks of *Zea mays*, Newfield, New Jersey, U.S.A., October 1878, J. B. Ellis; ex. Herb. R.B.G. Kew (slide filed as Herb. I.M.I. No. 8783). Type.

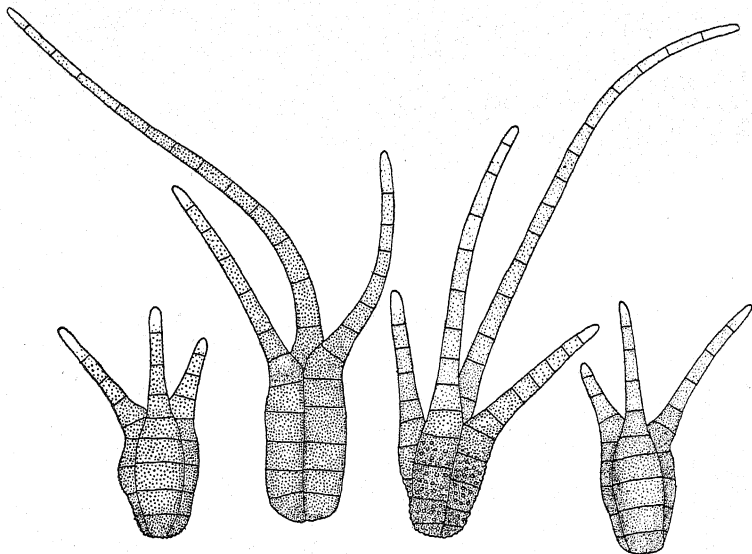


Fig. 2. *Tetraploa ellisii*, mature spores. ( $\times 500$ )

### 3. OTHER SPECIES OF *TETRAPLOA*

Little has been written about the other three species of *Tetraploa* and I have not seen specimens of them. *T. divergens* was found growing on *Panicum agrostidiiforme* [*P. laxum*] at Starkville, Miss., U.S.A., October 1894 and was described by Tracy and Earle (1895). According to their description the spores are about the same size as the *b* spores of *Tetraploa aristata* ( $12-14 \times 8-9 \mu$ ); the appendages however are very short ( $4-5 \mu$ ) and with-

out septa; there are usually three cells in each of the four columns. *T. scabra* is stated to have oval brown spores,  $30 \times 50 \mu$ , studded with minute papillae; the widely diverging septate setae are often ten times the length of the spore (Harkness, 1885); it was found on *Scirpus* at San Francisco, U.S.A. *Tetraploa muscicola* has been recorded only from Spain (Gonzalez Frago, 1916) on the liverworts *Aneura multifida* and *Lophozia quinque-dentata*. From their descriptions these species appear to be distinct from *Tetraploa aristata* and *T. ellisii*.

## SUMMARY

*Tetraploa aristata* is redescribed and an account given of its development, cultural characters, host range, spore dispersal and geographical distribution.

A description of *T. ellisii*, based on type material, is given; notes are made on three other species of *Tetraploa*.

I wish to thank Dr S. P. Wiltshire, Mr E. W. Mason, Dr G. R. Bisby and my brother Mr E. A. Ellis for kindly help and criticism.

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(Accepted for publication 1 March 1948)



## A BACTERIAL LEAF AND FLOWER DISEASE OF *ZINNIA* IN SOUTHERN RHODESIA

By J. C. HOPKINS AND W. J. DOWSON

(With Plate XXI)

An angular leaf spot of *Zinnia* was first observed in Southern Rhodesia about twelve years ago but was not reported again until March 1945, when both leaves and flowers of a large bed of plants growing at the Plant Pathological Laboratory in Salisbury were severely affected. Reddish and violet coloured varieties were more severely attacked than were those with yellow flowers.

Investigation showed that the disease was due to a bacterial pathogen, and the symptoms suggest that it may be the same as the *Zinnia* disease briefly described by Nannizzi (1929) in Italy. Nannizzi observed that the lesions were associated with bacteria often accompanied by *Trichoderma*, but he did not carry out any infection experiments with the bacteria, which he stated were spore-formers.

In Southern Rhodesia the disease first appears on the leaves as diffuse, yellowish, translucent, circular spots, 1–2 mm. in diameter. Under conditions of intermittent rainfall the spots slowly enlarge to about 5 mm. across and small reddish brown spots develop in the centres of the larger lesions, which continue to increase and become angular in shape. Each lesion is surrounded by a narrow margin of yellowish tissue, and if numerous, the lesions coalesce into rectangular areas 0.5–1.0 cm. long. Linear lesions may also form along the veins and develop into large dead areas involving most of the lamina. Generally, however, individual spots can still be recognized in the dead patches as angular areas varying in colour from dark brown to greyish white.

When atmospheric humidity is very high, there is a rapid expansion of the initial yellow spots. The dead areas quickly increase in size, coalesce, and if the humidity remains high, develop into ragged patches of decaying tissue. Under such conditions the disease may extend to the flowers and produce small brown spots on the ray florets. If infection is severe, the heads are seriously disfigured and may decay completely.

A yellow and a white bacterium were obtained from the lesions on the leaves and flowers in mixed culture, and were isolated in pure culture. Suspensions of each in sterilized water were atomized on to young *Zinnia* plants, but infection was obtained only with the yellow bacterium. Control plants remained healthy. The yellow bacterium was re-isolated and the infection experiments were repeated twice with positive results. Two other members of the Compositae, lettuce and burdock (*Arctium lappa*), were also inoculated in the same way, but neither was infected. Of the varieties of

*Zinnia* so far tested, Purple Prince, Brightness, Royal Purple and Lavender Queen are highly susceptible, the last-named being attacked in the stem, which may collapse. Yellow-flowered varieties develop mainly marginal infection, while red seem to be intermediate.

The *Zinnia* organism is a Gram-negative, rod-shaped bacterium with rounded ends and is provided with a single, long, polar flagellum. Yellow colonies are formed on most solid media. A honey coloured, slimy, abundant growth is produced on sterilized potato. Litmus milk becomes slightly alkaline and is peptonized without reduction of the litmus. Salicin is not attacked. The foregoing characters are those of the genus *Xanthomonas* (Dowson, 1939).

Two species of *Xanthomonas* are known to attack members of the Compositae: one, *X. vitians*, causes a wilting disease of lettuce in America (Brown, 1918); the other, *X. nigromaculans*, produces black spots on the leaves of *Arctium lappa* in Japan (Takimoto, 1927). The brief description of the last named available to us (Elliott, 1930) corresponds with that of *Zinnia* here recorded, and it is possible that the *Zinnia* bacterium may be a '*forma specialis*' of Takimoto's organism. The physiological characters and biochemical activities of *Xanthomonas nigromaculans* have not been fully investigated, and until these are known, or a culture is available, it is not possible to come to a definite decision as to whether or not the *Zinnia* pathogen is identical with that isolated from *Arctium*.

In view of the negative results obtained from the infection experiments with *Arctium*, of the fact that species of *Xanthomonas* are, in general, confined to a single genus of host plants, and also of the desirability of assigning a definite name to the *Zinnia* pathogen, we suggest that it should be regarded provisionally as a '*forma specialis*' of *Xanthomonas nigromaculans* (Takimoto) Dowson.

The following is a brief technical description of the *Zinnia* pathogen:

***Xanthomonas nigromaculans* forma specialis *zinniae* f. n.sp.**

Gram-negative rods with rounded ends, provided with one polar flagellum. Forming yellow colonies on meat-infusion or potato-glucose agar, and abundant, slimy, honey-coloured growth on sterilized potato. Hydrolyses starch. Does not attack salicin, reduce nitrates, or form ammonia from peptone. Produces hydrogen sulphide from peptone and liquefies gelatin. Slowly digests litmus milk which is rendered slightly alkaline, but does not reduce litmus. Forms acid in glucose, sucrose, maltose and lactose in peptone-free media.

Pathogenic in leaves and flowers of *Zinnia elegans*, forming yellow spots becoming brown.

Type locality: Salisbury, Southern Rhodesia.

Distribution: Southern Rhodesia and possibly Italy.

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## EXPLANATION OF PLATE XXI

- Fig. 1. Naturally infected leaves.  $\times 1$ .  
Fig. 2. Artificially infected leaves.  $\times 1$ .

(Accepted for publication 5 March 1948)

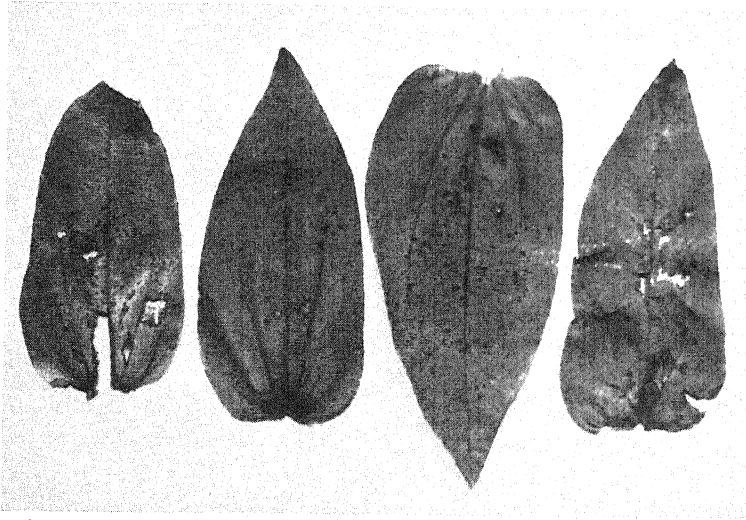


Fig. 1.

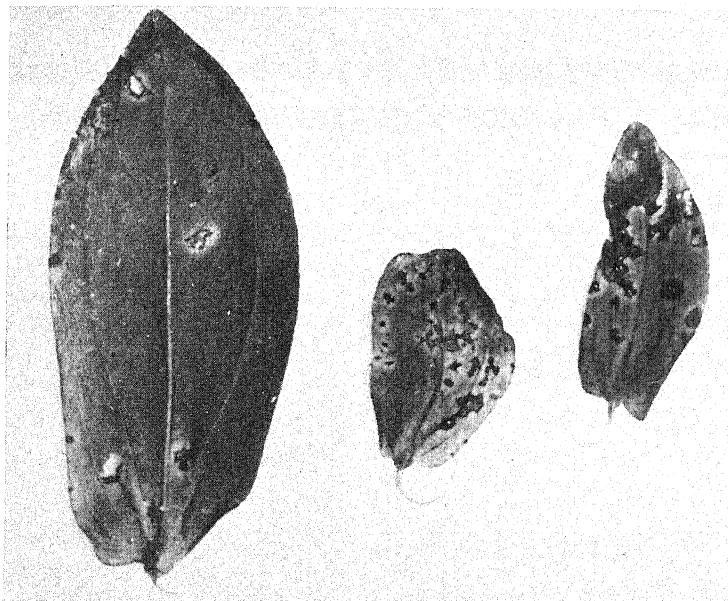


Fig. 2.



## THE GLADIOLUS SMUT

By G. C. AINSWORTH

*London School of Hygiene and Tropical Medicine*

During recent years the status of the gladiolus smut has been a matter of uncertainty. Dodge and Laskaris (1941), from their study of a 'Smut' disease of gladiolus in New York, concluded that the gladiolus disease previously reported from both Europe and North America as smut was not caused by one of the Ustilaginales, but by a species of *Papulaspora* which, without examining European material, they named *P. gladioli* (Requien) Dodge & Laskaris. The next year Hotson (1942*a*) described the results of a comparative study of material provided by Dodge and English material, from which he concluded there were two distinct diseases: one caused by a species of *Papulaspora* characterized by bulbils, 24–64  $\mu$  [28–64  $\mu$  in the histogram in his fig. 3] diam., with the average at 44  $\mu$ , composed of 2–8 central cells surrounded by a cortex of one or more layers of cells, the other by a species of *Urocystis* characterized by spore-balls, 14–23  $\mu$  [12–32  $\mu$  in the histogram] diam., each composed of one or two central cells surrounded by a single layer of cortical cells. He proposed the first fungus as a new species under the name *P. gladioli* Hotson (a later homonym for *P. gladioli* (Req.) Dodge & Laskaris and therefore invalid) and adopted *Urocystis gladioli* (Req.) Smith for the second. Six months later Hotson (1942*b*) described three more species of *Papulaspora* associated with decay in gladiolus corms and in the same paper *P. dodgei* Connors was proposed to replace '*P. gladioli* Hotson'. The bulbils of all these four species are appreciably larger than the spore-balls of the smut (see Hotson, 1942*b*, fig. 2).

In the autumn of 1941 I compared a specimen of '*P. gladioli*', kindly supplied by Dr B. O. Dodge, with the English collection (Herb. Path. Lab., No. 673) of which Hotson examined a part, and concluded, as did Hotson, that two species of fungi were involved. Search for the necessary type specimens could not be made under wartime conditions and only recently has the evidence needed to establish a name for the smut been assembled.

*Urocystis gladioli* W. G. Smith

The usually accepted designation for the gladiolus smut is *U. gladioli* (Requien) W. G. Smith, the name under which the species was compiled by De Toni in Saccardo's *Sylloge* (vii, p. 518, 1888). W. G. Smith (1876) did not, however, transfer Requien's species to *Urocystis*. He made no mention of the earlier species and wrote (p. 421): 'This new species I propose naming *Urocystis Gladioli*, and it may be characterized as follows: Sori (or clusters of spores in blisters) obliterated or effused, spores large, compound, consisting of from three to six inner brown cells, and a larger, indefinite number of nearly transparent outer cells, both series of cells

being fertile. Habitat—on and in the corms and scapes of *Gladioli*. (See fig. 84, enlarged 200 diam., and A, B, fig. 85, enlarged 1000 diam.)'

Search in the Herbaria of the British Museum (Natural History) and of the Royal Botanic Gardens, Kew, has failed to locate a type specimen, although at Kew there is a fragment of gladiolus corm labelled '*Urocystis gladioli* W. G. Smith' (without host, locality, or date) in W. G. Smith's handwriting. Careful examination of this specimen disclosed no spores. Smith reported that Dr Wittmack, Dr Magnus, and Dr Brefeld of Berlin had examined his preparations and considered that the compound spores belonged to *Urocystis*. He adds, 'Dr Brefeld says he has seen the *Urocystis* growing upon very different materials, even upon bread'. In my opinion there is no doubt that Smith's fungus was a species of *Papulaspora*. The 'spore-balls' as figured by Smith are 42–45  $\mu$  diam. (which approximates to the average diameter of those of *P. dodgei*); each is composed of five to six central cells surrounded by a cortex of transparent cells which, like the central cells, 'burst, and throw out threads of mycelium', all features more characteristic of *Papulaspora* than of *Urocystis*.

#### *Uredo gladioli* Requien

*U. gladioli* Requien was first described by Duby (*Botanicon Gallicum*, II, p. 901, 1830) as '*U[redo] Gladioli* (Requien in herb. DC.) bifrons, maculis circa lutescentibus, acervulis nigris suborbicularibus sparsis confertisque convexis epidermide bullatâ clausâ tectis, sporidiis subglobosis sessilibus reticulo pellucido tenuissimo obvolutis.—In Gladiolo circa Avenionem (cl. Req.).'

Again no type specimen has been located with certainty, but in the De Candolle Herbarium of the Delessert Herbarium, Geneva, there are two gladiolus leaves in the *Puccinia gladioli* Cast. folder which were collected by Requien, also at Avignon, in 1819. Through the courtesy of the Director of the Conservatoire et Jardin Botanique, Geneva, it has been possible to examine this specimen which, as the presence of teliospores proves, has been correctly classified. The original label attached to the specimen bears the words (in handwriting which may be Requien's) '*Xyloma? gladioli nob. Avignon*' with the addition by De Candolle of '*Mr Requien 1819*'. This specimen must have been diagnosed as *P. gladioli* subsequent to the description of the gladiolus rust by Castagne in 1842. It may well be the one on which Duby based *Uredo gladioli* Requien, especially as Linder (in Hotson, 1942*b*) records that there is a specimen of the telial state of the gladiolus rust in the Curtis Herbarium (Farlow Herb.) labelled by Curtis '*Uredo gladioli* Duby!' The first part of Duby's description suggests the rust, but the last four words are similar to those applied to *U. ranunculacearum* [*Urocystis anemones*] described on the same page. In the face of the above evidence the safest course is probably to reject *Uredo gladioli* Requien as a *nomen ambiguum* under Art. 62 of the International Rules of Botanical Nomenclature on the grounds that De Toni applied the name to a smut; Dodge and Laskaris (1941) used it for a species of *Papulaspora*, while Sydow (1904) listed *Uredo gladioli* Req. as a synonym of *Puccinia gladioli* Cast. The gladiolus smut is, therefore, now proposed as a new species.

***Urocystis gladiolicola* n.sp.**

*Sori* in the leaves, as dark brown blisters parallel with the veins 1 mm. to several cm. in length, at first covered by the epidermis which ruptures to expose the spores, and in the corms. *Spore mass* powdery, dark brown. *Spore balls* globose, 15–28  $\mu$  diam., each composed of one or two spores completely surrounded by a rather irregular layer of colourless sterile cells, 6–10  $\mu$  diam. *Spores* globose or slightly angled, reddish brown, 12–17  $\mu$  diam.

On cultivated gladiolus, Stoke Climsland, Cornwall, England. Collected by A. Beaumont, June 1944. (Herbarium Plant Pathology Laboratory, Harpenden, Herts, No. 14, Type.)

*Sori* foliicoli vel in cormis efformati, in foliis pustulas atro-brunneas cum nervis parallelis, 1 mm. ad plures cm. longas efficientes, primo epidermide tecti, dein erumpentes. *Massa sporarum* atro-brunnea, pulverulenta. *Glomeruli* globosi, 15–28  $\mu$  diam., e 1–2 sporis centralibus, cellulis sterilibus hyalinis, irregulariter dispositis 6–10  $\mu$  diam. circumdatis constantes. *Sporae* globosae vel leviter angulatae, rufo-brunneae, 12–17  $\mu$  diam.

Hab. in foliis cormisque Gladioli, Stoke Climsland, Cornwall, Anglia; leg. A. Beaumont, Jun. 1944. (Herb. Path. Lab., Harpenden, Herts, No. 14, Typus.)

Fragments of the type specimen (which has been figured by Moore (1948), figs. 25, 26) have been deposited in the Herbarium of the Royal Botanic Gardens, Kew and the Herbarium of the New York Botanic Garden.

Attempts made in November 1947 to germinate spores of the type specimen were unsuccessful.

I am indebted to the Keeper of the Herbarium of the Royal Botanic Gardens, Kew, and to the Keeper of Botany of the British Museum (Natural History) for allowing me access to the collections in their charge, to Mr W. C. Moore for his kindness in supplying specimens, and to Miss E. M. Wakefield for improving my Latin.

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(Accepted for publication 15 March 1948)



## NEW RECORDS AND OBSERVATIONS. IV

By A. A. PEARSON, F.L.S.

(With Plate XXII and 3 Text-figures)

This paper is divided into two sections. The first deals with species not previously recorded for Britain, but which were included in the Revised List of British Agarics and Boleti by Pearson and Dennis, published in these *Transactions*, vol. xxxi. The rest of the paper covers additions to the list and notes on uncommon or little-known species.

## SECTION I

**Lepiota serena** (Fr.) sensu Kühner. *Bull. S.M. Fr.* LI, 213.

*Pileus* 1-4 cm. fleshy, conico-campanulate with or without umbo, white, covered with silky radial fibrils, smooth or rimose-squamulose; margin smooth or appendiculate with veil. *Gills* crowded, white or creamy white, linear or ventricose, free, edge fimbriate. *Stem* 1-6 cm. long either equal or with bulbous base, sometimes strikingly ventricose, white, solid or narrowly fistulose. *Ring* superior, membranous, white. *Flesh* white or grey. *Taste* mild but rather rancid. *Smell* strong, pleasant. *Spores* white, elliptic-oblong,  $7-8 \times 3\frac{1}{2}-4\frac{1}{2} \mu$ , 1 guttulate, smooth without germinating pore, turn red in iodine. *Cystidia* on gill edge clavate or mixed with cylindrical cells,  $12-30 \mu$  wide.

*Habitat*: on ground or among dead leaves. N. Devon, September 1945 and October 1946. Wiltshire early November 1947. Very variable in size but easily recognized by the guttulate spores and rather voluminous cystidia.

There are several interpretations of *L. serena*. It was referred by Boudier to Cooke's Ill. 47 (57) of *Armillaria subcava* which is more likely to be *Lepiota brebissonii* Godey. The *L. serena* of Lange in *Fl. Ag. Dan.* 1, 29, Pl. 11 B has cystidia with a long beak and has been referred to *L. sericea* Cool by Huysman in his *Observations sur le Genre 'Lepiota'*: *Med. Ned. Myc. Ver.* xxviii.

*L. serena* seems to be fairly common in the west of England and has been sent to me on several occasions by F. R. Elliston Wright, Braunton, N. Devon, and T. W. Dunston, Donhead, Wiltshire.

**Lactarius representaneus** Britz. *Hym. a. Sudb.* (1885).

*Pileus* up to 16 cm., convex or flat and depressed, sometimes with small umbo, viscid, bright straw colour, violet when rubbed, glabrous in centre, elsewhere with adpressed scales; margin incurved and shaggy. *Gills* pale cream, thin, crowded, adnate or subdecurrent staining violet. *Milk* pale cloudy cream, unchangeable when isolated on a glass slide, but turning the flesh violet; taste like wild thyme then slightly bitter. *Stem* up to 10 cm. long, 25 mm. diam., hollow, subequal or slightly ventricose, somewhat

viscid or weeping when moist except at pruinose apex, then dry and glabrous, straw or golden yellow, pitted with shallow dull yellow hollows. *Flesh* pale cream, turning violet. *Taste* like the milk. *Smell* faint. *Spores* subspherical cristate echinulate  $9-11 \times 7\frac{1}{2}-9 \mu$ . *Cystidia* fusoid acute  $60-70 \times 10-12 \mu$ .

*Habitat*: in moist boggy places among leaves and moss. Found in Rothiemurchus Forest, Scotland.

This robust species is allied to *L. scrobiculatus* from which it differs in the flesh turning violet instead of yellow, also by the taste and brighter colour.

An excellent description and a beautiful coloured plate by Jules and Jeanne Favre are given in *Bull. Soc. Myc. Fr.* LV, Atlas, Pl. LXXXI (1939).

### **Russula rosea** Quél.

In these *Transactions*, XXII, 36 and XXIII, 307, *R. aurora* Krombh. and *R. rosea* Quél. were described separately, but the two descriptions should be combined and the least ambiguous of the two names would appear to be *R. rosea*. The range of colour is from a delicate pink to that of a ripe peach. The latter colouring is uncommon, while the pink form is fairly common and often recorded as *R. lepida*. The chemical reaction to sulphovanillin which turns the flesh of *R. rosea* to a brilliant carmine red is a useful and decisive diagnostic feature.

Many authors have used the name *R. aurora* for this species and it would have priority if it were certain that it is the same, but its identity is doubtful. Krombholz describes it as having a reddish stem and this suggests a form of *R. lepida*. In his Appendix II, *Trans. Brit. mycol. Soc.* xvii, 44, Carleton Rea has already recorded *aurora* Krombh. as a variety of *lepida* on the basis of Bresadola's description and figure, Tab. 414, in *Icon. Myc.* and this probably is a form of *R. lepida* but quite distinct from *R. rosea*. It is proposed for the present to adopt *Russula rosea* Quél. and record the peach-coloured form as forma *aurora* Pearson.

### **Entoloma turbidum** Fr.

*Pileus* 2-4 cm., fleshy, campanulate then expanded with obtuse or more rarely with acute umbo and undulate margin, radiately splitting when old, smooth, polished; almost black at first, then brownish or drab-grey (near Buffy Brown: Ridgway), darker in centre; margin inturned at first, cuticle slightly overlapping when expanded. *Gills* subdistant, grey, then pinkish grey, ventricose, broad, veined, adnate-emarginate then free, edge wavy. *Stem* 5-10 cm. long, thick set, sometimes even, but in typical forms clavate with very swollen base, often connate, rather firm, white with blackish grey striate fibrils, base white tomentose, stuffed with loose fibres. *Flesh* white. *Taste* mild. *Smell* faint or none. *Spores* in mass Vinaceous Cinnamon (Ridgway), subglobose apiculate with irregular outline but no distinct angles, contents granular-vacuolar  $8-9 \times 6\frac{1}{2}-7 \mu$  including apiculus. *Cystidia* absent. *Epicutis* of cap: septate hyphae  $7-9 \mu$  wide with greyish brown granular contents and hyaline clamp connexions.

*Habitat*: in mixed wood including birch. Brockenhurst, New Forest, Hampshire, 20 October 1947; also in Churt, Surrey, 14 October 1940.

The above corresponds to the type as described by Fries in *Monographia*,

p. 276, but as he there mentions, *E. turbidum* is found *sub plurimis formis*, many of which are not so clearly characterized as the New Forest specimens. The species figured by Lange in *Fl. Ag. Dan.* II, p. 96, Pl. 76H is quite different and suggests a large *Nolanea*.

***Naucoria arvalis*** Fr. and var. ***tuberigena*** Quél. 17 *Suppl. Ch. Jura* and *Vosges* (1889) as *Galera arvalis* var. *tuberigena* = *Naucoria sclerotina* Velenovsky in *Mykologia*, Prague, 1924, p. 144.

*Pileus* 1–2 cm., convex then flat, gibbous or with shallow umbo, somewhat fleshy in centre, membranaceous elsewhere, hygrophanous, dull ochre or honey colour or with a reddish tinge when moist, then bright ochraceous yellow, smooth, often rugulose with fine radiating veins, oily looking when moist, mat when dry, margin not striate with cuticle slightly overlapping gills. *Gills* crowded, pallid then brown adnate, more or less emarginate, linear, ventricose or wedge-shaped, edge straight or slightly wavy and fimbriate. *Stem* long and slender, 6–12 cm.  $\times$  2–4 mm. thick, white or pallid buff, darker below, tough and elastic with internal pith, equal or swollen below either with an attenuated rooting base or abruptly attached to a rugulose brownish black soft sclerotium 1–4 cm. *Flesh* honey colour in cap, paler in stem. *Taste* bitter. *Smell* none. *Spores* in mass snuff brown, elliptical with truncate apical pore  $9\text{--}10 \times 5\text{--}5\frac{1}{2} \mu$ , smooth. *Cystidia* on gill edge flask-shaped with pointed or blunt apex about  $40 \times 12 \mu$ ; on gill face club-shaped with 2 to 5 sterigmoid filiform appendages—mostly 3 in the specimens examined. Upper surface of cap hymeniform with sack-shaped cells mixed with fusoid cystidia.

*Habitat*: among débris of various kinds. Hindhead, Surrey, 30 September 1944, in garden. Near Haslemere, 9 September 1945, on rotting straw heap. Grayshott, Hampshire, among loose roadside sweepings.

The sclerotium was not seen in the first two gatherings, but in the last there were two fruit-bodies attached to a sclerotium the size of a walnut. This is much larger than the sclerotia hitherto recorded.

*N. arvalis* is probably fairly common and may be passed as a form of *N. semi-orbicularis* from which it differs in many particulars, notably in the bitter taste and remarkable cystidia on the gill face with their finger-like projections; also by the sclerotium which is not always present but may be overlooked and left in the ground.

There is a plate in Cooke's *Illustrations*, 506 (479), from a drawing by Worthington G. Smith which depicts specimens growing on sand at Yarmouth. Cooke labelled it *Agaricus (Naucoria) arvalis* Smith (not Fries). It probably is the same species. Agarics growing on the seashore often 'suffer a sea change'.

***Inocybe squamata*** Lange. Jakob E. Lange, *Dan. Bot. Ark.* Bd. 2, Nr. 7 (1917) and *Fl. Ag. Dan.* III, p. 83, Pl. 114A (1938).

*Pileus* 2–10 cm., convex, umbonate then flat and finally upturned with umbo obsolete or persistent, tomentose-arachnoid, olive buff with rusty patches, darker in centre (sec. Lange breaks up into scales on disk). *Gills* crowded, at first dingy yellow (Old Gold: Ridgway) then olivaceous brown with white fimbriate edge, adnate emarginate or almost free,

ventricose. Stem 3-7 cm. long, 5-10 mm. thick, stuffed with loose pith then hollow, olive-buff then reddish ochre, striately fibrillose with shaggy scales, equal or sub-bulbose, white tomentose at base. Flesh olive to ochraceous buff, ferruginous in pith of stem and with horny line above the gills. Taste mild. Smell none. Spore powder snuff brown. Spores elliptical subphaseoliform smooth  $8\frac{1}{2}$ - $9\frac{1}{2} \times 5$ - $5\frac{1}{2} \mu$ . Cystidia on gill edge densely packed, cylindrical or sack-shaped with thin walls. Epicutis of cap with hyphae 6-10  $\mu$  septate with small clamp connexions.

Habitat: gregarious; in moist land under oak, birch and willow, 2 October 1946.

The above description was taken from a large troop with caps ranging in size from 2 to 6 cm. wide, some with robust stems 7-10 mm. diam. They differ in the diagnosis of Lange by the absence of scales in the centre of the cap, but Lange may have seen old specimens only.

*I. squamata* must be rare as it is such a very striking fungus that otherwise it could hardly have been overlooked. It belongs to the group without thick-walled cristate cystidia and is close to *I. fastigiata* from which it differs totally in colour and has much smaller spores.

**Inocybe descissa** Fr. var. **brunneo-atra** Heim in *Le Genre Inocybe*, p. 234 (1931).

Differs from the type in the cap being radially streaked with dark brown almost black fibrils; often split showing white flesh underneath. Cystidia ventricose-fusoid with long neck and cristate apex  $50$ - $80 \times 18$ - $25 \mu$ . Spore powder snuff brown. Spores subamygdaliform, smooth,  $9$ - $10 \times 5$ - $6 \mu$ . Not uncommon.

Another fairly common variety is what Lange has described as *I. microspora* the spores being  $6\frac{1}{2}$ - $7 \times 4\frac{1}{2}$ - $5 \mu$ , but this is close enough to the original Friesian diagnosis of *I. descissa* to be considered the type, so for the present it may be left in the British list as such.

The following notes should be added to the 'Revised List':

*Pleurotus applicatus* (Batsch) Fr. has smooth globose spores  $4$ - $6 \mu$ . We have gathered it recently.

*Pleurotus applicatus* sensu Quélet, Rea non alt. = *P. silvanus* Sacc. This is the species with smooth elliptical bean-shaped spores  $7$ - $8 \times 3$ - $4 \mu$ .

*Crepidotus applanatus* (Pers.) Fr. has round warted spores  $5$ - $6 \mu$ . It has been retained in the list but requires confirmation.

*Crepidotus applanatus* sensu Quélet, Rea non alt. = *C. fragilis* Joss. (1939) = *C. autochtonus* Lange (1938). This is the species with elliptical or rather almond-shaped spores  $7$ - $9 \times 5$ - $5\frac{3}{4} \mu$ . The valid name is *C. fragilis*.

We owe the clearer elucidation of the above four species to M. Jossierand in *Bull. Soc. Myc. Fr.* XLIX, 360-4 and LIII, 218.

#### SECTION 2

**Mycena olida** Bres. *Fungi Trid.* 1, 73 (1887) and *Icon. Mon. Tab.* 240 (1928) = *M. trachyspora* Rea, *Trans. Brit. mycol. Soc.* XII, 216 (1927) = *M. gypsea* Lange non Fr. *Dansk. Bot. Ark.* Bd. 1, Nr. 5, 26 (1914) and *Fl. Ag. Dan.* II, 41, Pl. 52B (1936).

*Pileus* 5–20 mm., thin, conical, obtuse, rarely with acute umbo, sometimes striate by transparency, but usually opaque; white with slight yellow tint especially in centre; glabrous or delicately and innately fibrillose. *Gills* crowded, white, adnate-sinuate then free, linear or ventricose. *Stem* 1–3 cm. long, 1–2 mm. diam., white, smooth to the eye but minutely pubescent *sub lente*, equal or slightly attenuated above, tomentose at base. *Flesh* white. *Taste* mild. *Smell* none or faint of new meal when pressed between fingers. Gill edge more or less fertile with scattered projecting *cystidia* which are cylindrical or flask-shaped mostly with blunt apex  $40\text{--}60 \times 6\text{--}10 \mu$ . *Basidia* 2 or 4 spored. *Spores* in mass white, non-amyloid, ovate apiculate, smooth with granular contents or 1-guttulate, mostly about  $6\frac{1}{2} \times 4\frac{1}{2} \mu$  in British collections which had basidia with 4 sterigmata, but the full range is  $6\frac{1}{2}\text{--}9 \times 4\frac{1}{2}\text{--}5\frac{1}{2} \mu$  sec. Kühner.

*Habitat*: caespitose on the dead trunks or stumps of deciduous trees. Lessness Abbey Woods near Woolwich, November 1926, and 1927, on old elm stump, Churt; on beech stump 15 November 1947.

It is tempting to identify this species with the long-lost *M. luteo-alba* Bolton which, however, Fries associated with pine woods. *M. olida* may be more common than we have thought and has been overlooked because it appears on stumps in a small form very like *M. flavo-alba*. The larger form which Rea named *M. trachyspora*, through an incorrect observation of the spore, is rare in the south of England. It is said to be common in France where it occurs chiefly on horse chestnut, also on ash, beech, elm, poplar and sycamore.

Lange recorded this as a form of *M. gypsea*, a species which is closely related to *M. lactea* (Pers.) Fr. but with more definitely fusiform spores and less conical cap, which I have found growing in troops in a grassy avenue often attached to fragments of *Cupressus* twigs.

### ***Mycena clavicularis* Fr.**

*Pileus* 5–20 mm., dry, membranaceous, subglobose, campanulate or sub-conical, often with central papilla which flattens or collapses into a small central umbel; dingy white with black or brown striae reaching to the brownish disk. *Gills* crowded, smoky grey or greyish white with white edge, rather broad, ventricose or wedge-shaped, adnate with decurrent tooth. *Stem* 4–5 cm. long, 1–1½ mm. diam., hyaline or with smoky tint, smooth, polished, cartilaginous-elastic, very viscid. *Flesh* blackish in cap, white in stem. *Taste* mild. *Smell* none or faint. *Spores* in mass white, amyloid, oblong elliptic or obovate, apiculate,  $7\text{--}8 \times 5\text{--}5\frac{1}{2} \mu$ . *Cystidia* on gill edge densely packed with pyriform brush-like cells,  $10\text{--}15 \mu$ , set with warts.

*Habitat*: in troops under conifers; North Heath near Midhurst, Sussex, 23 October 1947.

This species is replaced in the British list and may be fairly common in coniferous woods after a downpour of rain. The stem is very sticky, but only the stem; there is no separable glutinous cuticle on the cap or filament on the gill edge as in *M. epipterygia* and other allied species of this group.

In these *Transactions*, xxii, p. 30, was described under the binomial *Collybia pseudo-clusilis* Joss. and Konr. a small agaric which at first I wanted to call *C. cessans* Karst., but deferring to the opinion of my old friend

Carleton Rea, it was identified with the first-named. Since then I have had occasion to reconsider this matter and think it desirable to withdraw *C. pseudo-clusilis* and replace it by *C. cessans* Karst. sensu Lange. The two species have some points in common, but as Jossierand (*in litt.*) has pointed out, *C. pseudo-clusilis* is of a gelatinous nature and quite distinct from our species. The points at issue have been further involved by the collection of another agaric of a remarkable gelatinous character, also very reminiscent of *C. pseudo-clusilis*, but with amyloid spores, which like the other two species occurs in the late autumn and winter. It has been suggested by both Kühner and Jossierand (*in litt.*) that this may be a grey form of *Omphalia maura*. This may be so, but it looks so very distinct from the type that further observations are called for. The usual habitat of *O. maura* is on burnt ground. It is possible that when it occurs elsewhere the whole aspect of the fungus may change.

**Collybia cessans** Karst. (1871 in *F. et Fl. Fenn. Notis.* xi, p. 219) sensu Lange in *Dansk. Bot. Ark. Bd.* 2, Nr. 3, p. 21 (1917) and *Fl. Ag. Dan.* ii, 17, Pl. 46A (1936).

*Pileus* 1–2 cm., convex then flat, depressed or umbilicate, pale grey with darker striae by transparence, soon opaque and when dry, silky silvery grey, polished, smooth. *Gills* distant, white or dingy white, ventricose, rather broad, emarginate with decurrent tooth, fairly thick with blunt edge which is smooth or minutely pubescent. *Stem* 1½–2 cm. long, 1–1½ mm. thick, grey, paler at apex, equal or slightly thicker above, polished. *Flesh* thin, pale grey drying to white. *Taste* mild. *Smell* none. *Spores* in mass white, non-amyloid, broadly oval to subglobose, apiculate, 5½–7 × 4½–5 μ, with granular contents or 1-guttulate. *Cystidia* on gill edge fringed with abundant projecting cells, cylindrical or slightly swollen below, 40–60 × 8–10 μ. *Epicutis* of cap with septate hyphae 6–10 μ diam.

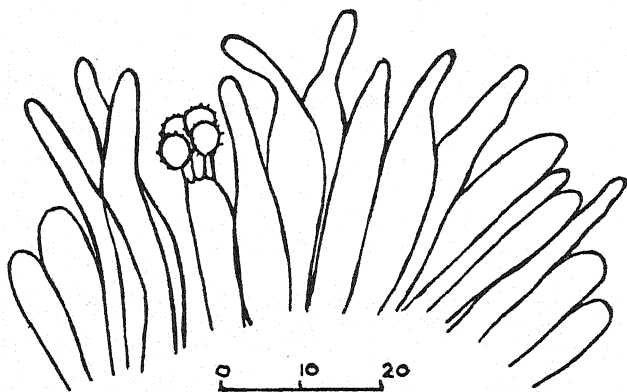
*Habitat*: on ground, usually in grass under conifers; solitary or in troops. Fairly common in November and December.

**Pleurotus palmatus** (Bull.) Fr. = *Rhodotus palmatus* (Bull. ex Fr.) Maire.

During the fungus foray of the Yorkshire Naturalists Union on 13 October 1947, several large specimens of this species were found growing on a stump near Jervaulx Abbey. The whole surface of the cap was ornamented with a network of raised veins. The opportunity was taken to verify some points. It has been stated that the spore powder differs in colour when a deposit is taken without the gills touching paper. Our specimens gave abundant deposits and both with and without contact of the paper the colour of the spores in mass was Pinkish Buff (Ridgway). This conflicts with the observations of René Maire and A. Pouchet, both of whom have stated that the colour is rusty brown when the deposit is made in contact with the paper. This may well happen when the specimens are so damp that the dirty moisture colours the spores, a not uncommon effect with any moist agaric when part of a spore print may be one colour and part another.

Pouchet, in *Bull. Soc. Myc. Fr.* XLVIII, 76–83, has given figures of the cystidia on the gill edge. These are not easy to observe. A microtome

section of the gill was prepared and confirmed Pouchet's observations. The edge is occupied by a narrow belt of very thin-walled fragile cystidia with densely staining protoplasm contents occupying even the tip of the 'beak'. The accompanying *camera lucida* drawing, kindly supplied by Dr R. W. G. Dennis, illustrates this.



Text-fig. 1. Edge of gill of *Pleurotus palmatus*

***Russula smaragdina* Quél. Suppl. 14, t. 12 (1885).**

*Pileus* 2-3 cm., convex, pale lemon yellow, mat, margin smooth without striae. *Gills* subcrowded, ventricose adnexed, white, edge smooth. *Stem* 3 cm. tall, 5 mm. diam., white, solid, equal. *Flesh* white. *Taste* mild (or with a faint acrid after-taste sec. Lange). *Spores* white in mass, globose or subglobose,  $6\frac{1}{2}$ - $7\frac{1}{2} \times 6\frac{1}{2}$ - $7\mu$ , reticulate all over, with no secondary finer network when seen under high magnification. Outline of spores with sixteen to twenty shallow projections. *Cystidia* on gill edge crowded, fish-shaped, mostly with blunt apex, turning dark blue all over with sulphovanillin; on gill face sparse; on cuticle of pileus numerous.

*Habitat*: oak woods, Derrycunnily Falls, Killarney, 3 September 1946. Rare.

Some authors have identified this small species with *Russula aeruginea* Lindbl., but in many of its characters it is quite distinct.

***Russula curtipes* Möller & Schaeffer. Bull. Soc. Myc. Fr. LI, 108-12.**

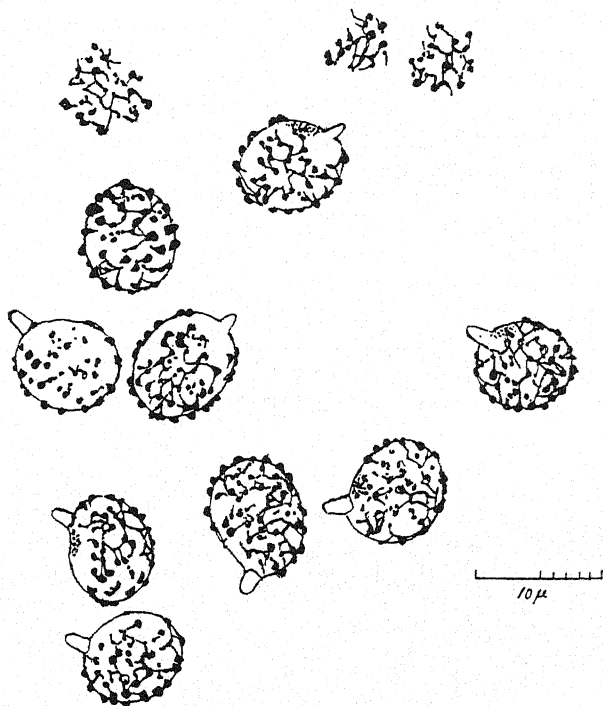
*Pileus* 5-12 cm., convex depressed, creamy buff or mottled with brownish red, deepening in colour towards margin which is reddish brown or purplish red, at first with a white tomentose or flocculose surface especially on disk, then smooth and mat, sometimes cracking; cuticle peels at margin which is only slightly striate-tuberculate when old. *Gills* crowded or subdistant, cream at first then the deeper ochraceous colour of the spores, spotted brown in parts, rounded in front, attenuated towards stem and minutely emarginate, anastomosing at base and often forked near stem. *Stem* 3-7 cm. long, 2-4 cm. diam., usually dumpy, white turning foxy brown when handled, often thicker above or below, pruinose or flocculose at apex, smooth or rather silky below, rugulose, solid and hard. *Flesh* white, com-

pect. Taste mild. Smell pleasant. Chemical reactions iron sulphate, flesh pink or greyish. Spores in mass ochraceous F of Crawshay's chart. Spores broadly oval with prominent apiculus, blunt warts and short or sometimes rather long crested ridges  $7\frac{1}{2}$ – $10 \times 6\frac{1}{2}$ – $8 \mu$ . Cystidia on gill edge not very abundant, obtuse or lanceolate, stain bluish black in sulphoranillin. Epicutis of cap with narrow vermiform hyphae only faintly staining blue in sulphoranillin, 4–5  $\mu$  wide.

Habitat: under beeches. Cowdray Park, Midhurst, Sussex, 3 and 22 August 1946.

This robust species may prove to be not uncommon. It has doubtless been confused with *R. alutacea* or *R. romellii* or even *R. xerampelina* from which it can be distinguished by the cristate spores.

Mr F. H. Møller, one of the authors of this species, kindly confirmed my determination of the specimens gathered at Cowdray Park.



Text-fig. 2. *Russula scotica* Pearson, basidiospores.

### ***Russula scotica* Pearson.**

In these *Transactions*, xxiii, 310, Pl. IX (1939), a new species of *Russula* was described and illustrated. The spores were drawn by me somewhat diagrammatically. Thanks to the kindness of Mr Richard Crawshay, I am able to give his *camera lucida* drawings of the spores of *R. scotica* which show their ornamentation much more accurately. These drawings will appear in the second edition of Mr Crawshay's book, *Spore ornamentation of the 'Russulas'*.



Highly magnified and in an iodine solution which stains only the armature of the spores, they are seen to have prominent warts and a rather coarse asymmetrical network, due to lines connecting the warts. The average size is  $8\frac{1}{2} \times 7\frac{1}{4} \mu$ .

**Entoloma cordae** Karsten = *Rhodophyllus cordae* (Karst.) Lange in *Fl. Ag. Dan.* II, 97, Pl. 76D.

*Pileus*  $1\frac{1}{2}$ –3 cm., rather fleshy, convex or flat with shallow umbo, grey with slight brown tinge drying to grey with black centre, silky, polished, not radiately fibrillose but minutely pubescent, striate when fresh, striate by transparence when fresh, then opaque. *Gills* distant, white then grey and finally with pinkish tinge, broad, ventricose, veined, adnate-sinuate then free. *Stem* usually tall, 5–6 cm. long, 4–6 mm. thick, white, striate, silky, equal or thicker below, often with pointed base, flexuose, fragile, hollow. *Flesh* dark grey in cap drying to dingy white and white in stem. *Taste* mild. *Smell* none or faint. *Spores* in mass Vinaceous Cinnamon (Ridgway), subglobose with prominent apiculus, outline only slightly irregular with granular vacuolar contents  $8$ – $10 \times 6\frac{1}{2}$ – $7 \mu$  including apiculus. Some of the spores are more definitely angular than others. *Cystidia* absent. *Cuticle* of cap with pale yellowish brown septate hyphae very indistinctly defined under the microscope.

*Habitat*: on ground under conifers; from Braunton, N. Devon, 25 October 1947. Under larch sent by Dr F. R. Elliston Wright; in pine wood, Great Common near Midhurst, Sussex, 6 November 1947.

This species belongs to what Romagnesi (*in litt.*) calls the 'turbidum' group which have small roundish subangular spores with vacuolar contents. It is, however, well-defined and worthy of specific distinction. From *E. sericeum* with which it can easily be confused, it differs in the absence of a mealy smell; the spores in the latter species are much more definitely angular.

**Naucoria muricata** (Fr.) Romagn. = *Pholiota muricata* Fr.

*Pileus*  $\frac{1}{2}$ –2 cm., rather fleshy, semi-globose, reddish brown, pulverulent with fugacious warts. *Gills* crowded, rather broad, clay colour then brown with white fimbriate edge, adnate, ventricose. *Stem* slender,  $1\frac{1}{2}$ – $2\frac{1}{2}$  cm. long,  $1\frac{1}{2}$ –3 mm. thick, covered with a fugacious mealy coating like the cap, reddish brown, paler and pruinose at apex. *Flesh* buff. *Taste* ? *Smell* pleasant. *Spore powder* brown. *Spores* pale yellow under microscope, bean-shaped, smooth,  $7$ – $8 \times 4$ – $4\frac{1}{2} \mu$ . *Cystidia* on gill edge with a fringe of flexuose hairs  $5$ – $6 \mu$  diam. with swollen tips  $9$ – $10 \mu$  wide. *Epicutis* of cap with globose or pyriform cells  $18$ – $20 \mu$ , surface slightly rough.

*Habitat*: in troops on old sawdust and chips of wood. Ross Island, Killarney, Eire, 1 September 1946. The normal habit is on fallen branches of deciduous trees.

The above looks very like *Naucoria siparia* Fr. which differs in the cap being covered with erect hairs built up from brown septate hyphae with a vaguely rough surface and clamp connexions. *N. siparia* I have gathered in Kent and it has also been sent from N. Devon. There are at least two other species with which it could be confused, notably *N. granulosa* Lange

which has not yet been recorded in this country, and *N. erinaceus* Fr. (= *Pholiota erinacea* (Fr.) Quél.) which turns up from time to time but has a much more scaly surface.

*N. muricata* is now replaced in the British List, but transferred to *Naucoria* in order to link it up with nearly related species.

**Naucoria scolecina** Fr. sensu Lange in *Dansk. Bot. Ark.* Bd. 6, p. 20 (1938) and *Fl. Ag. Dan.* iv, 21, Pl. 125 H (1939) = *Alnicola badia* Kühner in *Bull. Soc. Myc. Fr.* XLVII, 239 (1931) = *Tubaria umbrina* R. Maire in *Bull. Soc. Myc. Fr.* XLIV, 48 (1928).

*Pileus*  $1\frac{1}{2}$ – $2\frac{1}{2}$  cm., flat or with shallow umbo, hygrophanous, rusty bay or umber, smooth or with a furfuraceous pruinose surface, opaque or striate half way to centre. *Gills* subdistant, ventricose or arcuate, adnate or subdecurrent, concolorous, edge pallid. *Stem* slender, blackish or reddish brown, paler at apex, often white at base. *Flesh* dark brown when moist. *Smell* none or slightly raphanoid. *Taste* mild. *Spores* brown, almond or boat-shaped, minutely verrucose,  $9-11 \times 5-5\frac{1}{2} \mu$ . *Cystidia* on gill edge fringed with lanceolate cells having a bulbous base and long slender acute beak.

*Habitat*: in Alder swamps. Collected in Surrey and Sussex on several occasions.

The microscopic features of this species are practically the same as those of the common *Naucoria escharoides* which when fresh is a pale *café au lait*, but when old can easily be taken for the above. Lange has confused matters by stating that *N. escharoides* has elliptical spores, whereas they are pointed at both ends. The two species differ in colour, gill spacing and taste.

Neither of the epithets applied to the above species by Maire and Kühner are valid, both having been used elsewhere, but in a recent paper by Kühner (*Ann. Univ. Lyon*, 1942, p. 7) it is suggested that as another name has to be found, the one adopted by Lange should be accepted though what the original *Naucoria scolecina* was, none can say. Modern definitions of small agarics are largely based on microscopic observations which were not available in the days of Elias Fries.

**Galera clavata** (Velenovsky) Kühner in *Le Genre Galera*, p. 171 (1935) as *Galerina clavata*. Velenovsky in *Ceské houby*, p. 548, as variety of *Galera fragilis* Vel. non Peck (1921).

*Pileus* 1–3 cm., thin, obtusely conical or semi-globate, ochraceous, hygrophanous drying to buff, smooth, striate at margin or to disk.

*Gills* distant, broad, pale ochre or honey colour at first, ventricose, adnate-emarginate. *Stem* 3–7 cm. long,  $\frac{3}{4}$ –2 mm. thick, honey or ochraceous, equal, sometimes flexuose, pruinose above. *Flesh* pale ochre. *Taste* mild. *Smell* none. *Spores* in mass, rusty ochre (Antique Brown: Ridgway), elongate elliptical or obovate, with lateral apiculus  $12-14 \times 6-7$  ( $11-16\frac{1}{2} \times 6-8$ , 7  $\mu$  Kühner). *Basidia* with 2 or 4 sterigmata. *Cystidia* on gill edge skittle-shaped with long neck and globose apex, abundant or sparse. *Epicutis* of cap made up of septate hyphae 7–16  $\mu$ .

*Habitat*: in large troops on lawn, Cowdray Park, Sussex.

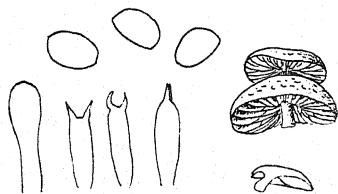
This species is characterized by the large spores and long-necked skittle-shaped cystidia.

**Galera nana** (Petri) Kühner.

A description of this species was given in these *Transactions*, xxvi, 48, but the spore measurements were omitted. They are almond-shaped minutely verrucose  $10-11 \times 5-6 \mu$ .

**Crepidotus hibernianus** sp. nov.

*Pileus* 5-15 mm., lateral, membranaceous, oblong shell-shaped, fibrillose-rimose, covered with small ferruginous scales; margin incurved and with traces of a white veil giving a denticulate appearance to the edge. *Gills* subdistant, narrow, linear, pallid buff then ochraceous or dark ferruginous, edge strikingly serrate-fimbriate. *Stem* rudimentary, obconical, ochraceous, pubescent. *Taste* mild or slightly bitter. *Smell* faint or none. *Flesh* light yellow. *Basidia* with 2 sterigmata,  $15-20 \times 4-5 \mu$ , oily contents reddish brown. *Spores* brown, broadly oval, smooth,  $8-10 \times 6-7 \mu$ . *Cystidia* on gill edge sterile with club-shaped cells  $6-7 \mu$  wide in tufts. *Epicutis* of cap formed of rather loosely woven thin-walled hyphae  $4 \mu$  thick, with reddish brown oily contents.



Text-fig. 3. *Crepidotus hibernianus* Pearson & Dennis. Basidia, spores and cystidia.  $\times 650$ .

*Habitat*: on log of *Tilia europaea*. Muckcross Park, Killarney, Eire, 29 August 1946.

Type specimens at the Herbarium, Royal Botanic Gardens, Kew.

This striking little species was gathered during a fungus foray at Killarney which Dr R. W. G. Dennis and I enjoyed in the genial company of several members of the Irish Naturalists Society.

**LATIN DIAGNOSIS:** *Crepidotus hibernianus* Pearson & Dennis. *Pileus* 5-15 mm. *lateralis*, *membranaceus*, *oblongulo-conchatus*, *fibrilloso-rimosus*, *minutis*, *squamulis ferruginosis*; *marginis involuto*; *velo marginato blanco denticulato*. *Lamellae subdistantes*, *angustae*, *lineares*, *pallido-fulvae deinde ochraceae vel obscuro-ferruginosae*; *acie valde fimbriato-serrulata*. *Stipes incohatus*, *obconicus*, *ochraceus*, *pubescentis*. *Caro lutea pallida*. *Odor subnullus*. *Sapor dulcis vel leviter amarescens*. *Basidia clavata* 2 *sterigmatica*  $15-20 \times 4-5 \mu$ . *Sporae fulvae*, *late ovaes*  $8-10 \times 6-7 \mu$ . *Acies lamellarum cystidiis clavatis*  $6-7 \mu$  *late*, *in fasciculis dispositis*. *Habitat*: *in trunco mortuo Tiliae europaeae*. Killarney, Hibernia.

**Crepidotus pubescens** Bres. *Icon. Myc. Tab.* p. 790 (1930) = *Dochmiopus terricola* (Britz.) Favre. *Schw. Zeitschrift für Pilzkunde* xiii, 147 (1935).

*Pileus* 1-3 cm., thin, white turning ochre when dry or pale lemon from the first, reniform with two round lobes, minutely pubescent, attached to host on back of pileus; margin incurved. *Gills* crowded or subdistant of several lengths, rather wide, ventricose, edge blunt smooth or minutely fimbriate, free, radiating from an eccentric point, white, turning pinkish and finally brownish. *Stem* absent or reduced to an excentric white papilla. *Taste* rather rancid. *Smell* none. *Spore powder* clay colour. *Spores* pale yellow under microscope, smooth, elliptic with lateral apiculus  $7-10 \times 4-4\frac{1}{2} \mu$  or cylindric subfusiform  $7-8 \times 3-3\frac{1}{2} \mu$ . *Cystidia* on gill edge with cylindrical

projecting cells, usually flexuose with blunt apex  $6-7\mu$  wide. *Epicutis* of cap made up of septate hyphae  $6-7\mu$  diam.

*Habitat*: on old leaves and stems especially bramble, nettle and bracken; very common.

This is the most frequent form in Britain of what hitherto we have called *Claudopus variabilis*. It appears, however, to be generally agreed that the specific epithet *variabilis* should be confined to the form with very minutely echinulate small spores about  $6 \times 3\mu$ . Bresadola's epithet has been adopted since Britzelmeyr's species is hardly well enough defined to call for its adoption.

It is a matter of opinion whether any of these forms deserve specific rank. The spore varies so much that on this character alone we could divide this little agaric into a long series based on spore shape and size.

The genus *Claudopus* should be confined to species with pink angular spores. French authors have adopted *Dochmiopus* Pat. for these small excentric agarics with what are usually described as pink spores. In a shallow spore deposit they do look pink, but when scraped into a small heap they are found to vary from light to dark clay colour or umber brown. There seems little justification for withdrawing them from *Crepidotus* in which group they were originally placed by Fries.

There is another type of angular or subangular spore which either has longitudinal ribs as in *Clitopilus prunulus*, or when the spore is looked at vertically, it is seen to have an irregular or polygonal outline with 7 or 8 sides, either obscurely angular or distinctly so. At least one species with this latter type of spore has the same field characters as *Crepidotus variabilis*; this is *Clitopilus pleurotelloides* (Kühner) Jossierand, which has not yet been recorded for Britain.

**Cortinarius (Hydrocybe) uraceus** (?Fr.) sensu Lange. *Fl. Ag. Dan.* III, 45, Pl. 102 B.

*Pileus* 3-5 cm., conical with acute or obtuse umbo, at first sooty black then hoary brown with a very slight purplish tint, radially fibrillose, shining; margin incurved, then straight or upturned, often rather ragged and splitting, with scanty traces of a white veil when young. *Gills* subdistant, horny coloured or fuliginous in very moist specimens, finally rusty dark brown, ventricose or wedge-shaped, adnate, emarginate, rather thick. *Stem* 6-7 cm. high, 4-6 mm. thick, concolorous, striate with silvery fibrils, but the whole stem having a distinct purplish red tint; equal or swollen at base, stuffed with loose fibres, soon hollow. *Flesh* horn colour near surface of both cap and stem, whitish in centre. *Smell* strong of new meal. *Taste* rancid. *Spores* in mass chocolate brown (Argus Brown of Ridgway), broadly ovate with short lateral apiculus, finely echinulate  $7-8 \times 5-5\frac{1}{4}\mu$ . *Cystidia* none.

*Habitat*: in troops under pine trees. Shiplake-on-Thames near Reading, 11 and 19 May 1947.

There are various interpretations of *C. uraceus*. The absence of an olive tint makes it doubtful if the above represents the species described by Fries, but for the present the name can be left unchanged.

**Cortinarius (Hydrocybe) junghuhnii** Fr.

*Pileus*  $1\frac{1}{2}$ – $2\frac{1}{2}$  cm., conical with acute or obtuse umbo, fleshy in centre, buff colour (Warm Buff: Ridgway) at first minutely scaly from the white veil, the scales usually persistent but sometimes wearing off except round margin. *Gills* fairly crowded; arcuate, ventricose or triangular, 3–4 mm. broad, brown with white fimbriate edge sometimes strikingly serrulate, adnate, emarginate with or without decurrent tooth; often anastomosing at base. *Stem* 3–8 cm. long, 3–4 mm. thick, somewhat firm and cartilaginous, hollow, buff coloured-like cap, fibrillose striate, equal or slightly swollen below, sometimes fusiform rooting. A vaguely defined white median ring may be present. *Flesh* buff. *Taste* mild. *Smell* none. *Spores* in mass reddish brown (Amber Brown: Ridgway). *Spores* pip-shaped with or without guttule,  $7-8\frac{1}{2} \times 4\frac{3}{4}-5\frac{1}{2} \mu$ , smooth or vaguely punctate. *Cystidia* on gill edge projecting in tufts, clavate of various lengths and diameters.

*Habitat*: under oaks near Gospel Green, Sussex, 25 September 1946.

Fries and Ricken give the habitat as in coniferous woods, Lange as under oaks, birches and pines. Lange also mentions the cystidia on gill edge. In our specimens all parts were buff coloured, but this may have been due to the dry weather prevailing at the time; normally the colour would probably be ochraceous cinnamon as given by Lange or pale brick colour as in the full description by Fries in *Monographia*, II, 113. The figures by Lange in *Fl. Ag. Dan.* Pl. 104E, only show the short-stemmed form, but among the large group gathered there were some specimens with a long fusoid stem as indicated by both Fries and Ricken.

**Stropharia ferrii** Bres. in *Riv. di Sc. Nat. 'Natura'*. Milan, XIX, 17 (1928) and *Icon. Myc.* Tab. 842 (1931) = *Stropharia rugoso-annulata* Farlow (1929).

In these *Transactions*, XXIX, 201, Pl. 30, a description and figures of *S. rugoso-annulata* Farlow were given. In a recent number of the Swiss journal *Schw. Zeitschrift für Pilzkunde*, January 1947, p. 12, A. Pilat has a note on a species found in Switzerland which he identifies with *S. ferrii* and synonymizes with *S. rugoso-annulata*. Another record from Switzerland was given by Métrod in *Bull. Soc. Myc. Fr.* LIII, 231–3 (1937).

The illustration by Bresadola gives an inadequate notion of the robust character of this species, but there seems little doubt that it is the same. It was first collected by Dr J. Ferri in northern Italy (Lombardy) and seems to be spreading. It is indeed strange that this very striking species first found in Italy, then in America, subsequently in Switzerland and England should have been overlooked until recent years.

**Psilocybe catervata** Massee.

*Pileus* 1– $2\frac{1}{2}$  cm., fleshy in centre only, campanulate-conical with obtuse umbo, white, when old with buff tint, minutely fibrillose *sub lente*, brittle. *Gills* subcrowded, umber brown with slight purplish tint, edge white fimbriate, linear or ventricose, adnate. *Stem* 3–4 cm. long,  $2-2\frac{1}{2}$  mm. thick, hollow but firm, white, striate with silky fibrils, equal or slightly swollen at base. *Flesh* white. *Taste* mild. *Smell* pleasant. *Spores* in mass sooty black, under microscope brown, elongate ellipsoid apiculate, truncate with germi-

nating pore  $10-12 \times 5-6 \mu$ . *Cystidia* on sterile gill edge ventricose with blunt or pointed apex  $10-25 \mu$  wide, mixed with sack-shaped cells. *Epicutis* of cap made up from short-celled septate hyphae  $20-35 \mu$  wide.

*Habitat*: on damp ground in troops; caespitose, near Jervaulx Abbey, Yorkshire, 13 October 1947.

**Boletus leoninus** Pers. *Myc. Eur.* (1825) = *Xerocomus leoninus* (Pers.) Quél.

*Pileus* 5-8 cm., convex then flat and depressed, dry, bright tawny or tan yellow with a slight sulphur or lemon tint here and there, changing to a pale reddish clay, covered with a close tomentum which is somewhat arachnoid, mat; margin incurved. *Tubes* and *Pores* wax yellow also with a very slight lemon tint, brownish when rubbed, adnate, emarginate, pores small, irregular. *Stem* lemon yellow above, dingy reddish brown below, ventricose, fusoid, minutely tomentose (*sub lente*), solid and firm. *Flesh* pure white almost unchangeable except for reddish tinge above the tubes and yellow near the surface. *Taste* mild. *Smell* strong, not unpleasant. *Spores* in mass Citrine (Ridgway), lighter in colour than the olive-green spores of many species; fusoid apiculate  $10-13 \times 5-5\frac{1}{2} \mu$ . *Cystidia* sparse, flask-shaped, hyaline.

*Habitat*: on ground of mixed deciduous woods including beech and birch; some pine trees not far away.

This was gathered by me at a Fungus Foray of the Bournemouth Natural History Society held at Brockenhurst, New Forest, Hampshire, on 20 October 1947. Two specimens were found from which the above description was made. *B. leoninus* has been recorded in the works of Micheli, Persoon, Krombholz, Quélet and Boudier, who in *Icones Mycologicae*, 1, Pl. 141 *bis*, has a coloured drawing all parts of which are tawny yellow except the flesh which is pale yellow—but his specimens were sent by post and we know what changes may take place in transit. He describes the cap as tomentose but cites Krombholz who states that the cap is completely naked. Persoon too says the cap is glabrous; Quélet that it is villous. In our specimens the cap (*sub lente*) was remarkably tomentose, but we cannot tell if this was partly due to the dry weather. A complete generalized description must wait till we know more about this very rare *Boletus*. I am indebted to E. J. Gilbert for help in identifying this species.

A rare *Hydnum*: During the Jubilee meeting of our Society a Fungus Foray took place at Windsor Park on 22 October 1946. In view of the cold weather prevailing at the time, little of interest was expected. However, several specimens of a creamy white *Hydnum* were brought in which perplexed all the experts present including our foreign visitors. These specimens were mostly without definite shape, with no pileus and only a few teeth in patches. Fortunately one good specimen was found with pilei clearly defined which I was able to take home. It proved to be *Hydnum diversidens*, a species which is not familiar to British mycologists. This may not be so rare as we have thought since it was brought in by several collectors, but in most cases it was growing in such inchoate shapeless masses that they would normally discard it as indeterminable. A description of the perfect example gathered is given below.

**Hydnum diversidens** Fr. = **Dryodon diversidens** (Fr.) Quél.

*Pileus* 9 cm. wide, 6 cm. thick, sessile, semicircular, flat or slightly depressed with obtuse but clearly marked margin, surface with blunt warts of irregular shape about 1 mm. long, cream colour (Cartridge Buff: Ridgway), pink when handled. Dried, the colour is tawny yellow streaked with orange. *Hymenium* concolorous with spines of various shapes, flat or round with blunt or toothed apices, mostly short, 1-2 mm., but longer ones present in the interstices. *Flesh* the same colour. *Taste* mild or slightly bitter after mastication. *Smell* not strong but pleasant. *Spores* white, subglobose or broadly elliptic, 1-guttulate, amyloid about  $4 \times 3 \mu$ . *Gleocystidia* abundant, long and flexuose, 4-6  $\mu$  wide, projecting like a fringe mostly with blunt apex, and with oily granular contents.

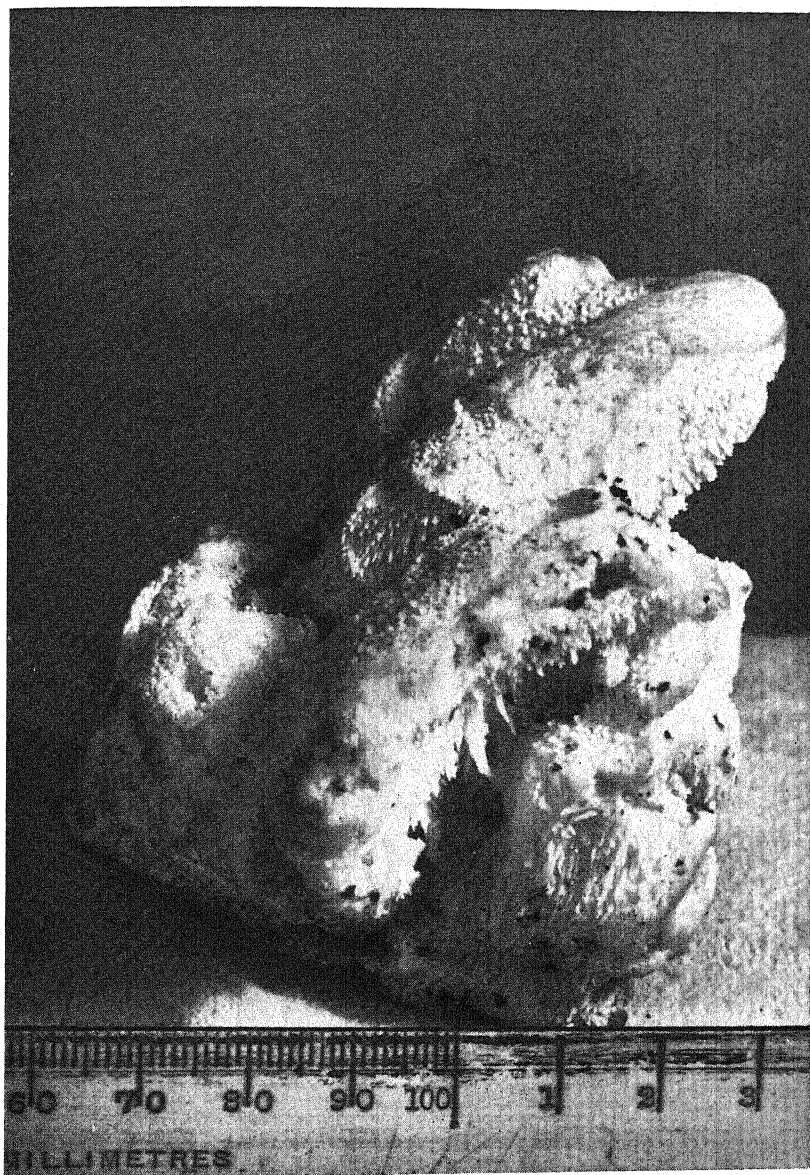
*Habitat*: on stumps in beech wood. Irregularly imbricate.

#### EXPLANATION OF PLATE XXII

*Hydnum diversidens* Fr. Specimen collected in Windsor Forest, 22 Oct. 1946.

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## NEW AND INTERESTING PLANT DISEASES

By W. C. MOORE AND F. JOAN MOORE

*Plant Pathology Laboratory, Harpenden, Herts*

## 37. TWO POWDERY MILDEWS ON ROCK ROSE

On 8 September 1947 Miss J. M. Gooby brought us some mildewed leaves from a seedling rock rose (*Helianthemum vulgare*) growing in her garden in Harpenden. Some half a dozen other rock roses nearby were unaffected. Within twenty-four hours the same mildew, which proved to be *Leveillula taurica* (Lév.) Arn., was found on several varieties of *Helianthemum vulgare* in three other gardens in Harpenden, and later the same month or in October it was found by us at Exmouth (Devon) and Luton (Bedfordshire), by Dr A. S. Boughey at Exeter, and by Mr W. Buddin on *H. rhodanthæ carnea* near Reading. Usually some varieties in the garden were affected, while others escaped: sometimes the mildew was found mainly on varieties with soft, broad leaves and sometimes on those with narrow, more wiry foliage. The fungus formed a dense white felt on the underside of the leaves and was also present to some extent, though much less conspicuously, on the upper surface. On microscopic examination, abundant branched mycelium was observed in the mesophyll and in the substomatal chambers. The conidiophores emerged singly or in twos or threes through the stomata, were simple or branched, and bore the conidia singly or in chains of up to six. The conidia were large and varied greatly in form, being long cylindric, elliptic, barrel-shaped or tapering towards one end. They measured  $45\text{--}70 \times 12\text{--}21 \mu$  (average of thirty spores  $56 \times 17 \mu$ ) and usually showed clearly a small scar of attachment. Many of them had germinated in situ, with germ tubes  $3 \mu$  wide and up to  $150 \mu$  long, developed from near one end of the conidium. Cleistocarps were found on some of the leaves, embedded and often hidden in the felt of mycelium. They were bright yellow when young, but soon turned brown and ultimately black. When examined at the end of September many of the cleistocarps were black, cauldron-shaped or pezizoid,  $90\text{--}200 \mu$  in diameter (average of thirty =  $130 \mu$ ), with numerous simple or branched mycelial-like appendages often with a knarled appearance, brown at the base, and hyaline at the tips. The contents were then not fully differentiated, but by the beginning of November, ripe ascospores were found in cleistocarps collected in Harpenden. The asci were not distinctly seen, but a number of ascospores, usually 8–10, occasionally more, measuring  $33\text{--}42 \times 16\text{--}19 \mu$  (average of ten =  $36 \times 18 \mu$ ), were present in each cleistocarp.

According to Blumer (1933), *Leveillula taurica* is mainly distributed in the arid steppe regions of central Asia around the Black Sea and around the Mediterranean. He listed it on *Helianthemum canum* (L.) Baumg. in Czechoslovakia, on *H. oelandicum* Koch. in Yugoslavia, and on *H. vulgare* Gaertn. in Poland. It has not been recorded before in the British Isles, and its

prominence in widely separated southern districts in 1947 may well have been closely related to the hot dry summer of that year.

On 10 November 1947, when examining plants of *Helianthemum* for *Leveillula* in the Royal Botanic Garden at Edinburgh, one of us (F. J. M.) found what appeared superficially to be this mildew on the undersides of the leaves of *Helianthemum pilosum purpureum*. On microscopic examination, however, the fungus proved to be different. The conidia corresponded in size and shape to those of most genera of the Erysiphaceae: they measured  $24-30 \times 15-18 \mu$  (average of twenty-five was  $29 \times 15 \mu$ ) and contained fibrosin granules. No cleistocarps were observed and the fungus could not be definitely identified, but it was perhaps the imperfect state of *Sphaerotheca fuliginea* (Schlecht.) Salm., which has been recorded (Blumer, 1933) on *Helianthemum vulgare* Gaertn. in France and Switzerland.

Material of *Leveillula taurica* is deposited in the Herbarium of the Plant Pathology Laboratory at Harpenden as Herb. Path. Lab. Nos. 1-4, and Nos. 429-30, and that of the *Oidium* on *Helianthemum pilosum purpureum* as Herb. Path. Lab. No. 6.

### 38. DOWNY MILDEW OF ROCK ROSE (*PERONOSPORA LEPTOCLADA*)

On 14 October 1947, while looking for *Leveillula taurica* at Bloxham, near Banbury (Oxon), Mr W. Buddin collected what he thought at first to be this mildew, on the undersides of the leaves of some seedling rock roses. On microscopic examination, however, he found the fungus to be a species of *Peronospora*, and he very kindly sent us the material for deposition in Herb. Path. Lab. (as No. 5). We identified the fungus as *P. leptoclada* Sacc. in Michelia II, p. 530, with conidia  $18-27 \times 18-21 \mu$  (average of thirty =  $24 \times 19 \mu$ ).

Gäumann (1923) distinguished two species of *Peronospora* on *Helianthemum*. One of these, found on *H. vulgare* Gars. and *H. guttatum* Mill., with conidia averaging  $26 \times 19 \mu$ , he identified with *Peronospora leptoclada*; the other, on *Helianthemum alpestre* (Jacq.) Dunal. and *H. niloticum* Mönch., with more spherical and somewhat larger conidia averaging  $27.8 \times 23 \mu$ , he named *Peronospora alpestris* Gäum. Nothing is known about the biological behaviour of these two fungi on the various species of *Helianthemum*, and the morphological differences between them seem to be very slight.

### 39. POWDERY MILDEW ON CHERRY LAUREL

Moore (1943) has referred to the occasional occurrence of an *Oidium* on cherry laurel (*Prunus lauro-cerasus* L.) in South Wales. Mr John Rees noticed a rather striking instance of it at Cardiff in July 1937, on a cherry laurel hedge. The hedge, which was continuous along the frontage of two houses, was almost wholly affected in front of one house and almost completely unaffected in front of the other. The difference was thought to have been related to a difference in the date of clipping the two parts of the hedge. In September 1938 slight attacks of the same mildew were seen again in various parts of Cardiff, as well as at Usk (Monmouthshire). In November of the following year, when the mildew developed on a number of frontage hedges in one road in Cardiff, Mr Rees kindly sent one of us (W. C. M.) specimens of it (Herb. Path. Lab. No. 28). The mildewed leaves

were twisted and ruffled, and the upper surfaces showed indefinite chlorotic areas, which made the affected hedges look conspicuous. The undersides of the leaves were partly covered with a sparse web of white or pinkish white mycelium. There were also some light reddish brown roughened areas on these leaves, but similar areas were found on a few leaves that showed no evident sign of the mildew. The conidia were borne in chains and when freshly mounted in water were cylindric, rounded at both ends, contained fibrosin bodies, and measured  $30-41 \times 12-17 \mu$  (average of fifty =  $34 \times 15 \mu$ ). No cleistocarps were seen.

Powdery mildew is evidently not common on cherry laurel either here or abroad, and its identity is still uncertain. Cleistocarps have not been observed at all in Britain, and when Salmon (1906) previously found the *Oidium* here in 1905 on *Prunus lauro-cerasus* and its var. *camelliaefolia* in the Royal Botanic Gardens at Kew, he only assumed that it belonged to *Sphaerotheca pannosa*, chiefly because he found the persistent pannose patches of mycelium characteristic of that species on the young stems. Bertolini (1879) had been the first to describe an *Oidium* on this host: he observed it on cherry laurel fruits in Italy and named it *O. passerinii*. The following year, Roumeguère (1880) recorded *O. passerinii* from Tarbes in France, but he claimed to have found cleistocarps as well, each with four asci and five to seven brown spores, and he named the fungus *Erysiphe bertolini*, though according to Salmon (1906) it was distributed in Roumeguère's Exsiccati Fung. gall. No. 963 under the name *Oidium lauro-cerasi*. The mildew has also been identified—probably correctly—as *Podosphaera oxycanthae* (DC.) de Bary var. *tridactyla* (Wallr.) Salm. Fischer (1919)\* found both conidia and cleistocarps of that fungus in Switzerland. The cherry laurel bushes in the Botanic Garden at Berne were pruned hard following damage by frost during the winter of 1918-19, and mildew appeared in September 1919 on the new and still immature shoots that had developed during the summer. Fischer explained the comparative rarity of the disease on cherry laurel on the assumption that at the time when the *Podosphaera* usually appears on other species of *Prunus*, cherry laurel leaves are normally fully developed, hard, and unsusceptible.

#### 40. ALTERNARIA STEM BLIGHT OF GODETIA (ALTERNARIA GODETIAE NEERGAARD)

Early in September 1947 a few dry, almost leafless, and apparently dead godetia plants were received at the Plant Pathology Laboratory from Feering, near Kelvedon in Essex. These plants were said to be suffering from a disease that had appeared rather suddenly two or three weeks previously, and that had progressed rapidly through certain stocks raised for seed purposes, particularly those of the varieties Sybil Sherwood and Kelvedon Glory. The specimens showed no obvious sign of a root disease or of having wilted, and their appearance suggested that they might be suffering from the effects of the severe summer drought that had prevailed. The stems were more or less blackened by a coating that consisted chiefly

\* An article by van der Lek (1920) on mildew of cherry laurel merely summarizes Fischer's observations.

of the conidiophores and conidia of a species of *Alternaria*, whilst *Botrytis cinerea* was fruiting on the seed pods, buds, and remains of the petals, but both these fungi were regarded at the time as probably secondary. Pycnidia of *Diplodina passerinii* Allesch. were also noticed on one or two stems, though the typical symptoms of the disease caused by this fungus were lacking.

On 22 September one of us (W. C. M.) visited the affected field with Dr R. E. Taylor, who a few years previously had studied Wilt of godetia caused by *D. passerinii* (Taylor, 1941). The seed crop from a bed of Kelvedon Glory, about fifty yards by ten, was being harvested that day, though the yield was a very poor one, for most of the plants were prematurely brown and dry, the leaves had either fallen or they fell off as soon as they were touched, and the fruits contained little or no mature seed and were shed early. It was not difficult to find pycnidia of *Diplodina* on some of the plants, but the typical symptoms of Wilt, notably the characteristic rot at the base of the stem, were lacking. Nor did it appear probable that the premature withering of the plant was solely an effect of drought in what had been a hot and sunny summer. A few specimens, together with a sample of seed collected from withered plants, were taken back to Harpenden for closer examination. The same species of *Alternaria* as had occurred on the original specimens was present on the dead stems in soot-like patches consisting mainly of conidiophores, often fasciculate, bearing chains of spores. Most of the spores were beaked, and the beak sometimes terminated in a swollen tip. The total length of the spores, including the beak, ranged from  $27$  to  $66\mu$  (average  $46\mu$ ): the body measured  $18-48 \times 9-18\mu$  (average of fifty =  $34 \times 13\mu$ ) and the beak was  $0-30\mu$  long (average  $12\mu$ ). The spores showed  $3-8$  transverse and  $0-6$  longitudinal septa. In tube and plate cultures on potato-dextrose agar there was at first a copious, white, aerial mycelium which soon became greyish green, while the submerged hyphae gradually became dark greenish black. Spores from cultures were very variable in size and most of them were unbeaked.

Thirty of the seeds brought back from Feering were placed on moist filter-paper in a dish on 29 September, and were examined about a fortnight later. Five of them failed to germinate: all these became covered with the *Alternaria* and on two of them *Botrytis cinerea* Fr. developed in addition. The rest had germinated, but all showed signs of disease, which either began from the hypocotyl or from a leaf to which the testa had become attached. Some were already dead, and none grew to be more than an inch high before succumbing. The *Alternaria* developed on or was isolated from twenty seedlings, while *Botrytis cinerea* was found on seven and cultured from nine seedlings.

Some months later we received commercial samples of seed of the two varieties Kelvedon Glory and Sybil Sherwood, said to have been harvested from infected and non-infected crops in the nursery at Feering. Seventy seeds of each variety from both crops were tested in a Copenhagen germinator. The seeds and seedlings were examined after eighteen days and the results obtained are shown in Table 1. The *Alternaria* developed on most of the seeds that failed to germinate, and also severely attacked many of the young seedlings produced. The seed from the crops not noticeably

affected in the field was contaminated with the fungus, but only to about one-half the extent of the seed from the obviously infected crops.

The species of *Alternaria* concerned was identified as *A. godetiae* Neergaard (1945*b*). This is the species which Neergaard (1938) described, under the name *Alternaria* sp., as the cause in Denmark of a disease obviously identical with that seen in 1947 in Essex. Neergaard noticed this disease first in 1937 and for the next few years it was severe at times on seed crops of godetia in Denmark (especially on the varieties Kelvedon Glory and Sybil Sherwood), often with the result that the yield of seed was reduced to a small fraction of the normal. In his monograph on Danish species of *Alternaria* and *Stemphylium*, Neergaard (1945*a*) named the fungus *Alternaria tenuissima* (Fr.) Wiltsh. var. *godetiae* n.var., but later (1945*b*) he raised it to specific rank as *A. godetiae*. He demonstrated the pathogenicity

Table 1

	Seeds failing to germinate (%)	Seeds developing <i>Alternaria</i> (%)	Seedlings becoming infected (%)
Kelvedon Glory:			
Crop obviously infected	17	46	33
Crop not obviously infected	3	23	23
Sybil Sherwood:			
Crop obviously infected	8	47	40
Crop not obviously infected	6	27	24

of the fungus (1945*a*), showed it to be carried on the seed (1938-45), and obtained some measure of control by seed disinfection for half an hour in a  $\frac{1}{4}$ % solution of Uspulun (1938). Incidentally, Neergaard (1938-45) has also found *Botrytis cinerea* on godetia seed on a number of occasions, and has shown that it can be pathogenic to sterile seedlings and mature plants.

*Alternaria* Stem Blight has not hitherto been seen in any country other than Denmark.

#### 41. STEM ROT OF MELON (*MYCOSPHAERELLA CITRULLINA*)

About forty years ago, Grossenbacher (1909) investigated a disease of muskmelon (*Cucumis melo* L.) which had caused a good deal of damage in the greenhouses of the New York Agricultural Experiment Station, and he proved that this disease was caused by a fungus which he named *Mycosphaerella citrullina*. About the same time Massee (1909) received specimens of a stem rot of tomato from Waltham Cross and a similarly diseased cucumber plant from Gloucestershire. He found on both plants a species of *Ascochyta* which he regarded as identical with the imperfect stage of Grossenbacher's fungus, and as in a few preliminary inoculation experiments he apparently succeeded in infecting young tomato plants with spores taken from the cucumber plants, to say nothing of vegetable marrow with spores from tomato, he did not hesitate to attribute both diseases to *Mycosphaerella citrullina*, though he saw only the pycnidial state. Others (see e.g. Brooks & Price, 1913) followed Massee's example for a time, but ultimately Brooks and Searle (1921) showed that at any rate the tomato

fruit rot, which had by then become widely distributed in England, was caused by the fungus now known as *Didymella (Diplodina) lycopersici*. Among the many strains of *Phoma*, *Ascochyta* and related fungi which they studied, was an authentic culture of *Mycosphaerella citrullina* (C. O. Smith) Grossenb. sent to them by Dr C. L. Shear in 1919, as well as two herbarium specimens of *M. citrullina* collected on cucumber fruits and water melon in Florida, and herbarium material collected in England under the same name from tomato and melon. After a very careful study of the different strains Brooks and Searle concluded that 'contrary to the usually accepted idea, the species *Mycosphaerella citrullina* (C. O. Sm.) Gross. has not yet been found in England'. They suggested that it should therefore be deleted from the list of known British species because 'the cultural characters and general behaviour of authentic specimens of *Mycosphaerella citrullina* from America do not agree with any of the fungi isolated from tomato in this country'. This view was generally accepted, the name *M. citrullina* ceased to be used in this country for a parasite of tomato and cucurbits, and the species does not find a place in the latest list of British Pyrenomycetes (Bisby & Mason, 1940).

Nevertheless, recent observations by us indicate that this fungus does occur in Britain. On 10 July 1947, Mr E. Skillman sent us a portion of the stem of a diseased melon grown at Hockley, Essex. The plant was one of a number thought to be suffering from a disease which had troubled the same grower some thirty years previously. Uniformly and thickly distributed over the piece of stem were numerous partially or wholly immersed, black, spherical perithecia, 135–200  $\mu$  in diameter (average of thirty-five = 170  $\mu$ ), each with a clearly marked ostiole 24–27  $\mu$  across. The asci were fasciculate, paraphysate, unthickened at the apex, 45–63  $\times$  9–12  $\mu$  (average of twenty-five = 57  $\times$  9  $\mu$ ), and each contained eight hyaline ascospores. These were usually monoseriate, two-celled, with the distal cell nearly always larger than the other, oval with rounded ends, rather markedly constricted at the septum, and measuring 9–18  $\times$  3–6.5  $\mu$  (average of seventy = 13  $\times$  4.5  $\mu$ ). Many spores were top-shaped, with the larger cell broadly ovate and the smaller tapering. Mixed with the perithecia were a number of what we took to be pycnidia, though we could find no spores in them.\* They were somewhat irregular in shape, lighter in colour than the perithecia, and ranged in size from 85  $\times$  75 to 128  $\times$  110  $\mu$  (average of twenty = 113  $\times$  96  $\mu$ ).

This fungus agrees very closely with the description and figures of *M. citrullina* (C. O. Smith) Grossenb., with which we identify it. We have compared it with material of the perfect state of *Didymella lycopersici* as collected by Hickman (1944) on tomato at Long Ashton (Herb. Path. Lab.

\* In material from the same batch of plants examined at Cheshunt, Mrs Sheard found pycnidia measuring 91–128  $\times$  87–120  $\mu$  (average 115  $\times$  105  $\mu$ ) and containing two-celled spores 9–13  $\times$  4–6  $\mu$  (average 11  $\times$  4  $\mu$ ). In a few preliminary inoculation experiments Mrs Sheard obtained a rot of cucumber fruits but not of tomatoes with the fungus from melon, and she successfully infected tomatoes but not cucumber fruits with *Diplodina lycopersici* from tomato. Shortly afterwards she examined cucumbers affected with a stem rot caused by a fungus similar to that on the melon, and with this fungus successfully inoculated both cucumber and melon.

No. 96) and, apart from the aparaphysate nature of the perithecium which is a distinguishing feature of *Mycosphaerella*, there are distinct differences in the size and shape of the ascospores. Those of *Didymella lycopersici* are longer, more spindle-shaped and less constricted at the septum.

*Mycosphaerella citrullina* is not restricted to melon. Abroad, mainly in U.S.A. and Japan, it has also been held responsible (see, for example, Wiant, 1945) for a wilt or fruit black rot of *Cucumis sativus* L. (cucumber), *C. melo* L. (musk melon), *Citrullus vulgaris* Schrad. (water melon), *Cucurbita maxima* Duchesne (winter squash), *C. pepo* L. (summer squash, gourd, pumpkin, vegetable marrow), *C. moschata* Duchesne (Cushaw squash), and *Sechiumedule* (Jacq.) Swartz (chayote).

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## THE EFFECT OF ADDING TRACE ELEMENTS TO CZAPEK-DOX MEDIUM

By GEORGE SMITH

*Department of Biochemistry, London School of Hygiene and  
Tropical Medicine*

Culture media containing only well-defined, chemically pure substances are widely used for studying fungi. For taxonomic work their chief advantage is their reproducibility by workers in different laboratories, whilst for biochemical studies of all kinds they are obviously more suitable than more complex media made from plant and animal extracts, even though the latter may be essential for maintaining vigour in stock cultures of many species.

Probably the most generally used synthetic medium is that devised by Czapek and modified by Dox, containing, per litre, sodium nitrate 2.0 g., potassium chloride 0.5 g., crystalline magnesium sulphate 0.5 g., potassium phosphate (either  $\text{KH}_2\text{PO}_4$  or  $\text{K}_2\text{HPO}_4$  according to the pH required) 1.0 g., crystalline ferrous sulphate 0.01 g., and any desirable source of carbon. In this laboratory Czapek-Dox medium, containing 3 % sucrose and 1.5 % agar, as recommended by Thom (1930), has been used for many years in routine examinations of species of *Aspergillus* and *Penicillium*, and the same basal medium but with 5 % glucose as source of carbon has been used for numerous biochemical studies. However, during the late war (there is no record of the exact date, but, for reasons given below, it was probably the summer of 1941) the agar medium suddenly changed in character, although, as far as could be ascertained, it was still made up from the same brands of pure chemicals, and from the same batch of agar, a large quantity of which had been purchased prior to the war. It was found that many species of *Penicillium* began to grow atypically, producing semi-floccose, pale brownish colonies, with tardy and sparse production of conidia. Sporing usually commenced only at the edges of colonies which had grown to fair size over a period of five or more days.

All the constituents of the medium were tested for possible toxic impurities, but nothing was found which could explain the change. Next a batch of medium was made up with tap water and tested alongside the usual medium made with distilled water. There was no appreciable difference between the two media with any of a large number of species tested. Similarly, additions of yeast extract or corn-steep liquor affected the growth of very few species.

It was then recollected that, prior to 1941, all the distilled water used for making culture media came from an old tinned copper still which had been in continuous use since 1929, and that it had frequently been reported from various departments of the School that the water contained traces of copper. The still was completely destroyed in May 1941 and a more modern type, subsequently installed, produced a distillate in which no trace of copper

could be detected. It was therefore decided to test the effect of adding copper and other trace elements to the ordinary Czapek-Dox medium.

(a) *Addition of copper and zinc.* Four batches of medium were made, one containing no trace elements (except the usual iron), one containing 0.001 %  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , one with 0.001 %  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , and the fourth with the same percentages of both salts. A number of species of *Penicillium*, all of which had been found to grow abnormally on the usual medium, were sown simultaneously on all four media. Both copper and zinc, used separately or together, had a marked stimulatory effect on spore production. In general, copper alone was better than zinc alone, and the combination, for a few species, better than either element used singly.

(b) *Other trace elements.* As a preliminary the mixture of trace elements used by Brian, Curtis and Hemming (1946), including iron, copper, zinc, manganese and molybdenum, was tried. There was slight stimulation of some species, but in general the results were much inferior to those obtained with copper and zinc in Exp. 1. The concentrations of trace elements in this mixture are apparently too low for maximum effect, iron and zinc being approximately 1/10, and copper 1/70, of the amounts used in Exp. 1.

(c) Five media were made and tested simultaneously on a number of species of *Penicillium*. They contained trace elements as follows: (1) Brian's mixture at ten times the recommended concentration; (2) similar but with increased copper; (3) as (2) but with addition of calcium as chloride; (4) copper and zinc alone; (5) none.

The actual percentage amounts of salts of trace elements in the four modified media, omitting iron which was present at the same concentration in all five media, were:

	(1)	(2)	(3)	(4)
Copper sulphate	0.00015	0.0005	0.0005	0.0005
Zinc sulphate	0.001	0.001	0.001	0.001
Manganese sulphate	0.0001	0.0001	0.0001	—
Potassium molybdate	0.0001	0.0001	0.0001	—
Calcium chloride	—	—	0.001	—

With all the species used cultures on (2), (3) and (4) were almost indistinguishable, and a great improvement on the cultures on ordinary Czapek-Dox. With some species, but not all, medium (1) was as good as the other trace element media, but in no case was it better. The results indicated that copper and zinc are the essential trace elements and that additions of small amounts of manganese, molybdenum and calcium have little if any effect.

(d) In order to find whether copper and zinc, used in the comparatively high concentrations as in medium (4) above, have any adverse effect, this medium was used for the routine subculturing of a collection of about 250 isolates of species of *Penicillium*. In no case was there any evidence of a toxic effect and with some sixty species there was marked improvement in freedom of spore production. The same medium was then used for growing several hundred species and strains of other genera of moulds. In many cases there was definite stimulation, and only with a few strains of yeasts was there indication of a slight toxic effect.

(e) In view of the observed effects of copper and zinc on spore production it was obviously of interest to test the effect of these elements on yields of metabolic products, since it has often been noted in these laboratories that the best yields of characteristic products are associated with rapid and typical growth. Very encouraging results have already been obtained with two species of *Penicillium*.

*Citromycetin*. This substance, first described by Hetherington and Raistrick (1931*a*), is produced by most strains of *P. glabrum* (Wehmer) Westling. This species was chosen because it had already been found that most strains grow atypically on ordinary Czapek-Dox and are markedly stimulated by additions of copper and zinc. Citromycetin is readily detected in culture fluids by adding ferric chloride solution, this giving a very intense green colour. With some strains of the mould the ferric chloride reaction, after about two weeks' incubation, is so intense that the solution, contained in an ordinary test-tube, is quite opaque and has to be diluted many times before becoming transparent. The relative amounts of citromycetin produced under different cultural conditions, or by different strains of the mould, may be roughly estimated by comparing the dilutions required to bring the solution to some arbitrary standard depth of colour.

Seven strains of *P. glabrum* were used for the experiment. Each strain was sown in four flasks of ordinary Czapek-Dox solution containing 5 % glucose (hereafter denoted as C-medium) and four flasks of the same medium to which was added 0.0005 % copper sulphate and 0.001 % zinc sulphate (CT-medium). The flasks were of one litre capacity and each contained 350 ml. of medium. All the flasks used for one strain were sown from as nearly as possible identical slopes and were incubated at 24° C. Flasks were harvested after five, eight, eleven and fifteen days' incubation.

Growth on CT-medium was much more rapid than on C-medium, as was also the consumption of sugar. For example, after eleven days' incubation residual glucose in the CT-medium varied, for different strains, from 1.3 to 1.6 %, and on C-medium from 3.0 to 3.9 %. On C-medium none of the strains gave, at any stage of incubation, more than a faint reaction for citromycetin. On CT-medium the worst strain gave a ferric chloride colour which was only just transparent, whilst the other six strains gave reactions ten to seventeen times as intense.

*Citrinin*. This yellow pigment was first isolated from *P. citrinum* Thom by Hetherington and Raistrick (1931*b*). It is also produced in good yield by *P. implicatum* Biourge (author's unpublished observation). It is readily isolated, as it is precipitated almost completely on acidification of the culture fluid. *P. implicatum* was sown on C- and CT-media (as described above), thirty flasks of each medium. It had been found previously that the yield of citrinin reaches a maximum when the apparent residual glucose (by polarimeter) has fallen to between 0.5 and 1.0 %. The cultures on CT-medium were harvested after nineteen days' incubation, when the apparent residual glucose was 0.66 %. The culture fluid, on acidification, gave 20.0 g. of good quality citrinin. The cultures on C-medium showed considerable variation. Of two flasks tested after nineteen days' incubation one had 1.13 % residual glucose and gave no precipitate on acidification, whilst the other had 1.32 % glucose and gave a fair amount of precipitate.

The cultures were finally harvested after twenty-seven days and gave only 5.9 g. of crude citrinin which was somewhat brownish and of inferior quality to that obtained from the CT-medium.

#### SUMMARY

1. Some moulds, particularly a number of species of *Penicillium*, grow atypically on Czapek-Dox medium made up with pure chemicals.
2. The medium is satisfactory if copper (0.0005 %  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and zinc (0.001 %  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) are added.
3. Addition of copper and zinc stimulates production of citromycetin by *P. glabrum* and citrinin by *P. implicatum*.

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A NEW PREDACIOUS SPECIES OF *TRICHOTHECIUM*

By C. L. DUDDINGTON

*Biological Laboratories, The Polytechnic, Regent Street, London*

(With 2 Text-figures)

In June 1947, in the course of investigations on the distribution of predacious fungi in decaying vegetable matter, a species of *Trichothecium* was found to be capturing and destroying nematodes in considerable quantity.

The fungus first appeared when sterile plates of maize-meal agar were inoculated with small quantities of vegetable compost supplied by Dr M. C. Rayner from composts prepared under her direction at Bedford College, London. A few days after inoculation it was observed that a number of rather straight, septate hyphae, mostly  $3-5\mu$  in diameter, were growing out from the inoculum over the surface of the agar. These hyphae bore at intervals short lateral branches which, by curving back and anastomosing with the main hypha or with other similar branches, formed a series of three-dimensional networks (Fig. 1a). The loops were usually  $35-65\mu$  in external diameter and  $25-40\mu$  in internal diameter, and the hyphae which formed them contained dense cytoplasm with numerous large vacuoles. The loops were very similar to those formed by *Arthrobotrys oligospora* Fres., as described by Zopf (1888) and others.

Nematodes moving over the surface of the agar were captured by adhesion to these loops, and in struggling to escape the animals often became entangled in the meshes of the networks, though such entanglement was by no means necessary for effective capture. For a short time the nematode would struggle violently, but in all observed instances they were held fast and, usually after about an hour, became still and apparently dead. It seemed clear that the nematodes were held by some sticky substance, though on no occasion was it possible to observe the presence of the viscous fluid which is often to be seen when eelworms are captured by *Arthrobotrys oligospora*.

Shortly after capture, while the nematode is still alive, its cuticle is penetrated by a short outgrowth from the loop to which it adheres, the outgrowth originating from the point of contact. This outgrowth expands within the nematode to form a bulbous structure (Fig. 1b), usually  $10-23\mu$  in diameter, from which trophic hyphae, usually  $3.5-5.5\mu$  wide, grow out to fill the carcass of the eelworm and absorb its contents, so that eventually only the integument, filled with hyphae, remains. At first these trophic hyphae contain abundant cytoplasm, but later, when their work of absorption is completed, most of the cytoplasm is withdrawn.

A notable feature of the predacious activity of this fungus was the avidity with which nematodes were captured and consumed. They were often caught in groups of as many as a dozen, forming masses easily visible to the naked eye, and almost completely obscuring the networks which

held them. A number of different species were captured, some of them of large size; the biggest measured was a specimen of *Rhabditis* 1.2 mm. long. The networks themselves, however, were often of comparatively small extent; on the whole they were less extensive than those normally formed by *Arthrobotrys oligospora*. Often some of the loops of a network stood perpendicular to the surface of the agar-like croquet hoops, a position which probably added to their efficiency.

The actual cause of death of the captured nematode is uncertain. The bulbous structure intruded by the fungus soon after capture does not itself seem to be sufficient to kill the animal in the rather short time observed between capture and final cessation of movement. This infection bulb seldom measures more than two-thirds of the diameter of the eelworm, and often less than half. Drechsler (1937) states that in the retiary series of predacious Hyphomycetes this 'mortiferous excrescence'—to use his terminology—is directly responsible for the death of the animal by cutting it physiologically in half. After extensive observations of *A. oligospora*, *A. musiformis* Drechsler and the fungus at present under discussion, I have found nothing to substantiate this view. It appears to me more probable that the primary function of the bulbous intrusion is to provide a surface from which trophic hyphae can grow out quickly and in quantity into the interior of the animal. Whether any toxic substance is produced by the fungus is a matter for experiment to determine, but in the absence of further knowledge of the functions of these structures, to refer to them as 'mortiferous excrescences' seems apt to be misleading. The term 'infection bulb' would appear more in accordance both with what is so far known of them, and with the original terminology of Zopf (1888).

After eelworms have been extensively captured over a period of several days, conidia are produced, usually very sparingly. The conidia are borne in terminal clusters at the apices of erect conidiophores, 50–200  $\mu$  long. The conidiophores are often branched, sometimes more than once, a head of spores being formed at the apex of each branch. At first a single conidium appears as a swelling at the apex of the conidiophore; this then bends over to one side and a second conidium is formed at a point on the conidiophore morphologically just below the first. This process is repeated several times, so that a cluster of ten or even more conidia may be carried on one conidiophore (Fig. 2a).

The conidia are ovoid, usually 27–37  $\mu$  long and 14–16  $\mu$  in greatest diameter, with a broadly rounded distal end and a very bluntly rounded proximal end, with no trace of any apiculum. They are usually divided by a transverse septum into two approximately equal cells, and are absolutely sessile (Fig. 2b). They germinate readily, germ tubes often being produced by both cells.

In addition to conidia, intercalary chlamydospores are formed in large numbers, especially in older cultures. These are usually nearly spherical, 24–32  $\mu$  in external diameter, but sometimes ellipsoidal, measuring 28–59  $\mu$  long and 15–29  $\mu$  wide. These chlamydospores have a wall about 2  $\mu$  thick, composed of at least two layers. Under laboratory conditions they are known to have remained viable for six months, germinating readily at the end of that time when placed on moist agar.

The fungus was isolated in pure culture by picking off conidia under the microscope, and made good growth on maize-meal agar and on glucose Czapek's agar, the growth on Czapek's agar being improved by the addition of organic nitrogen. In pure culture the hyphae grow in rather straight

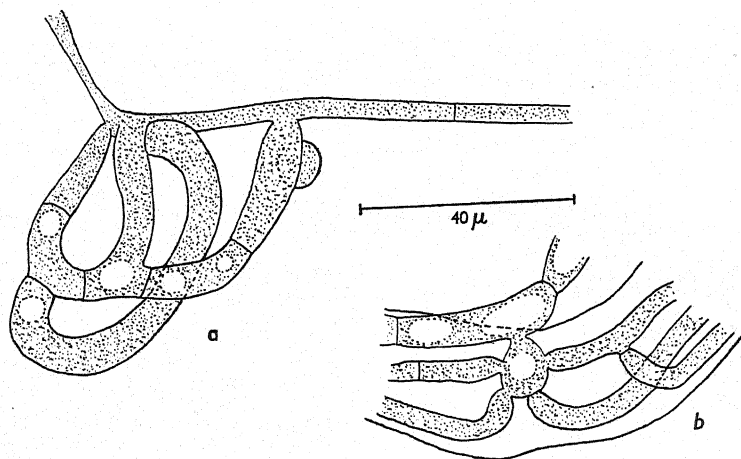


Fig. 1. *Trichothecium flagrans*. *a*, hyphal network; *b*, infection bulb within portion of eelworm, showing origin of trophic hyphae.

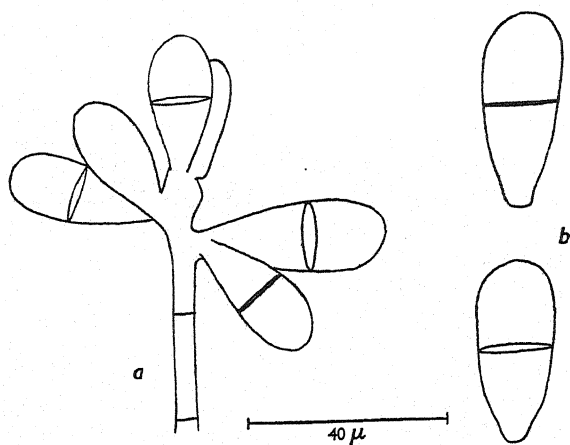


Fig. 2. *Trichothecium flagrans*. *a*, apex of conidiophore, bearing a cluster of six conidia; *b*, detached conidia.

lines radiating from the point of inoculation, producing anastomosing lateral branches of somewhat smaller diameter than the main hyphae, but no loop systems. On adding living nematodes to such a culture, the networks are quickly formed, and eelworms are captured. Chlamydospores are produced in large numbers in pure culture, but formation of conidia is

very limited, and declines progressively during a series of transfers from plate to plate. Sporulation may be induced by culturing on rabbit-dung agar, even after the power to produce conidia has been apparently lost.

The morphology of this fungus, and especially the organs of capture, are suggestive of *Arthrobotrys*, but the shape of the conidia and the manner in which they formed in succession on conidiophores without sterigmata are more characteristic of *Trichothecium*. It is accordingly described as a new species of that genus, under the binomial *Trichothecium flagrans*, the epithet being chosen on account of the savagery with which it captures and consumes eelworms, as well as the large size of some of the nematodes with which it is able to cope.

***Trichothecium flagrans* sp.nov.**

Mycelium expansum, hyphis (2) 3-5  $\mu$  latis sparse ramosis, spatiis 25-95  $\mu$  septatis; ramis lateralibus curvisque ad principem hypham atque inter se conjunctis qui reticula tri-dimensionis formant; laqueis singularibus (25) 35-65  $\mu$  latis extus, (12) 25-40 (47)  $\mu$  intus; nematoda adhaesa in laqueis implicata capiens; bulbo infectionis 10-23  $\mu$  in diam. intrudente per integumentum nematodis e quo hyphae edentes 3.5-5.5 (6.5)  $\mu$  latae excrescunt ad corpus implendum atque ad substantiam nematodis absorbendam. Conidia hyalina, continua effecta, quae caput 10 plus ad apices conidiophorum, saepe ramosorum, formant; ad altitudinem 50-200  $\mu$  supra substratum, 5.5-8.5  $\mu$  latum fundo, 3.5-5 (7)  $\mu$  apice. Conidia sessilia, (20) 27-37 (45)  $\mu$  longa, (11) 14-16 (17.5)  $\mu$  in max. diam., apice proximo late globoso, (3.5) 4-6 (7)  $\mu$  lato, plerumque per septum in duas partes circa aequas diviso. Mycelium etiam chlamydospores intercalarios efferens, plerumque subglobosos 24-32  $\mu$  in diam., subinde ellipsoideos plerumque (18) 28-59  $\mu$  longos, (15) 18-29  $\mu$  in diam.

Nematoda generis varii mensuraeque usque ad 1.2 mm. capiens atque edens. Hab. in retrimento plantarum e Bedford College, London.

Type material has been deposited in the Herbarium of the Commonwealth Mycological Institute, Kew, and in the Herbarium, Royal Botanic Gardens, Kew.

I should like to express my gratitude to Dr M. C. Rayner for supplying the sample of compost from which the collection was made, to Mr R. A. Gillanders for assistance in preparing the Latin diagnosis, and especially to Dr B. Barnes for valuable help at all stages of the work.

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# **OIDIOPSIS GOSSYPHII (WAKEF.) RAYCHAUDHURI** **F. INDICA F.NOV. ON COTTON**

By S. P. RAYCHAUDHURI

*Division of Mycology and Plant Pathology, Indian Agricultural  
 Research Institute, New Delhi*

(With 2 Text-figures)

In December 1941, *Gossypium barbadense* Linn., grown at Dacca, was found to be affected by powdery mildew and about 11 % of the plants were infected with the fungus. Ashy white mycelial patches were observed on either side of the diseased leaf which turned yellow before leaf-fall. The disease was observed only on older leaves. The infection generally appeared late in the season and the diseased plants produced very few flowers and bolls.

The mycelium is hyaline, mostly superficial and borne on both sides of the leaf. Only a few hyphae penetrate the leaf through the stomata. These hyphae measure  $4.5-6\mu$  in diameter. The conidiophores are simple, erect, septate, bearing broadly elliptical conidia. The conidia (Fig. 1) are vacuolate, granulate and borne singly at the tip; they measure  $31-41 \times 19-25\mu$  with an average size of  $37.5 \times 23.8\mu$ . Perithecia were not observed.

Microscopical examination of sections of diseased leaves has revealed that the mycelium penetrates through the mesophyll tissue on both sides of the lamina (Fig. 2).

Mildew of cotton has been recorded by several workers. Nowell (1923) records *Ovulariopsis gossypii* as causing a leaf mildew of Sea Island cotton in the Lesser Antilles. Butler (1918) reports the occasional occurrence of *Oidium* sp. on cotton in Bombay and suggests that the disease may be identical with the mildew *Oidiopsis taurica* (Lév.) Salm. (= *Erysiphe taurica* Lév.) of guar (*Cyamopsis psoraloides* DC.), while Abbott (1932) has expressed the opinion that *Ovulariopsis gossypii* is the imperfect stage of *Erysiphe malachrae* Seaver.

Zaprometov (1930) describes *Leveillula taurica* Arnaud f. *gossypii* on the upper surface of the leaves of two varieties of cotton, e.g. (i) Egyptian cotton 'Nubari', and (ii) upland type 'Navrotsky's Triumph' from Russian Central Asia. On the former variety of host the disease at its first appearance is distributed in separate angular portions 1.5-2 mm. in diameter which later coalesce into a continuous tomentose covering, while on the latter, the white tomentose mealy layer covers the leaf in separate irregularly rounded scattered spots. The conidia measure  $43-50 \times 13\mu$  and the perithecia have a diameter of  $157-175\mu$ .

Wakefield (1920), however, found only the conidial stage of the fungus on Sea Island cotton (*Gossypium barbadense* Linn.) and named it *Ovulariopsis gossypii* Wakefield. The conidial measurements as found by her are  $50-60 \times 16-22\mu$ . In a personal communication she writes that the Indian fungus is a distinct form from that described by her, in having shorter and

broader conidia. Moreover, she is now of the opinion that the fungus should be placed under genus *Oidiopsis* and not *Ovulariopsis* as described by her in 1920. Arnaud (1921) defined *Ovulariopsis* Pat. & Har. as being the conidial form of *Phyllactinia*, and characterized by having a single terminal club-shaped conidium and mycelium for the most part superficial. *Oidiopsis* Scalia he distinguished as the conidial form of *Erysiphe taurica*, for which he created the new genus *Leveillula*. In this fungus the mycelium is developed chiefly within the leaf, only after showing some external mycelium.

The Indian form of the fungus showed only the conidial stage. No perithecia were found on the host. The conidia differed from those of *Leveillula taurica* Arnaud f. *gossypii* in being shorter in length and broader in width.

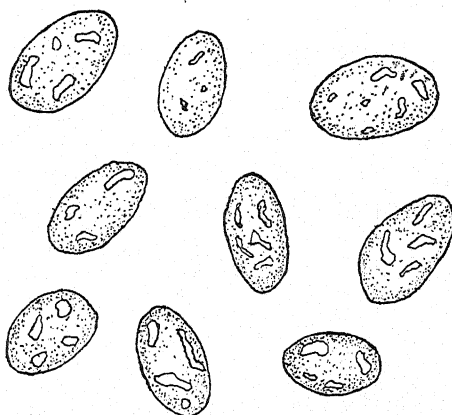


Fig. 1

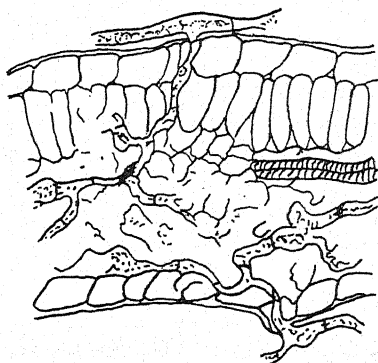


Fig. 2

Fig. 1. Conidia of *Oidiopsis gossypii* (Wakef.) Raychaudhuri f. *indica* f.nov.  $\times 380$ .

Fig. 2. Transverse section of a part of infected cotton leaf showing the mycelium of *O. gossypii* (Wakef.) Raychaudhuri f. *indica* f.nov. within the mesophyll tissue.  $\times 48$ .

In the light of Miss Wakefield's communication and as suggested by her, a new combination for the conidial form is proposed, e.g. ***Oidiopsis gossypii*** (Wakef.) Raychaudhuri nov.comb. Due to the marked difference in conidial measurements of this fungus a new form is proposed. The fungus is hence named ***Oidiopsis gossypii*** (Wakef.) Raychaudhuri f. *indica* f.nov.

*Latin diagnosis.* Mycelium hyalinum, fere omnino superficiale, in utraque facie foliorum; nonnullae tantum hyphae e stomatibus exeuntes; hyphae  $4.5-6\mu$  diameter. Conidiophora simplicia, erecta, septata, late ellipticis conidiis ornata. Conidia vacuolata, granulata, apicalia, solitaria,  $37.5 \times 23.8\mu$  ( $31-41 \times 19-25\mu$ ). Hab. in foliis maturis *Gossypii barbadensis* Linn. Dacca, Bengal, December 1941, leg. S. P. Raychaudhuri (Typus). Typus in herbario Kewensis.

I am grateful to Miss E. M. Wakefield, Kew Herbarium, for taking much interest in the diagnosis of the fungus and to Dr R. W. G. Dennis, Royal Botanic Gardens, Kew, for sending a photostatic copy of a portion

of Zaprometov's paper. My thanks are due to Rev. Father H. Santapau of St Xavier's College, Bombay (now in England) for writing the Latin diagnosis.

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# THE *BOTRYTIS* DISEASE OF *GLADIOLUS* WITH SPECIAL REFERENCE TO THE CAUSAL ORGANISM

By J. W. L. PEIRIS, PH.D.

The work to be described in this paper arose as a sequel to the investigations of Dr L. E. Hawker of the Plant Pathology Laboratory, Imperial College, who in the years 1944 and 1946 published three papers relating to various fungus diseases of gladiolus. The last of these papers (Hawker, 1946) dealt with the *Botrytis* disease. The object of the present investigation was to explore the latter more fully, with special reference to the identity of the causal organism.

## PLANT MATERIAL AND GENERAL EXPERIMENTAL METHODS

This investigation was begun in 1945 with a stock of about 1200 corms left by Dr Hawker from her experiments and consisting mainly of 'Lilac Wonder', a rather robust and prolific variety. There were also small quantities of the varieties 'Van Tienhoven', 'Wolfgang', 'Flaming Sword', 'Rose Precoce', etc. suitable only for small experiments. Although it was thought desirable to have carried out experiments with a number of varieties, it was not possible under the existing wartime conditions to supplement the continually dwindling stocks and most of the work had to be confined to the variety 'Lilac Wonder'.

The corms were grown and harvested at the Field Experiment Station, Slough, and stored in an unheated shed over winter. They were cleaned two or three weeks after harvesting unless otherwise stated, and placed in shallow trays during storage. The diseased corms in the experimental lots were recorded at intervals of approximately ten days during storage. The healthy corms were planted out in March–April in the usual manner and the conditions of growth and flowering observed.

The experiments consisted of isolations from diseased plant material, inoculation of plants in the field and in storage, lifting and cleaning corms at different periods, and storage under various conditions.

## EARLIER ACCOUNTS OF THE DISEASE

The *Botrytis* disease of gladiolus was first reported in 1927 from Canada by Drayton (1928), and observed in the same year by Moore (1939) in England. From then onwards it has been reported as follows: by Drayton (1929) from Holland in 1929; by Van Poeteren (1938) from the same country with a statement that it was becoming more prevalent; by Dodge and Laskaris (1941) from Long Island, N.Y.; by Noble, Hynes, McCleery & Birmingham (1935) as attacking stems and leaves in New South Wales; by Dimock (1940) as causing a leaf and flower blight in Florida. The three most recent works on the subject are by Timmermans (1941, 1942) in Holland, Wade (1945) in Australia and Hawker (1946) in England.

In many of these papers there was no attempt to identify the causal fungus critically. There was no doubt of the presence of a *Botrytis*, but no specific determination was made. Some authors assumed it to be *B. cinerea*. Klebahn (1930) was the first to study the fungus in some detail. His conclusion was that it was morphologically different from *B. cinerea* and accordingly he named it *B. gladioli* Kleb. Klebahn's paper, however, contains no description of the disease. The later work of Timmermans (1942) showed that the causal fungus was different from the one described by Klebahn; hence the new species *B. gladiolorum* Timmermans. Hawker's work, it should be added, was concerned mainly with the control of the disease; she assumed the fungus to be *B. cinerea* and stated that it was similar in spore size to the fungus described by Moore (1939).

#### SYMPTOMS OF THE DISEASE

Although the *Botrytis* rot of gladiolus is most conspicuous and becomes most destructive on the corms during storage, it is, nevertheless, capable of attacking the plant at any stage of its life history. The following account gives my experience of the disease manifestations and is in substantial agreement with that given by Moore.

(a) *Symptoms during early growth.* The commonest cause of gaps in the rows is the planting of corms affected by *Botrytis*. Such affected corms may be passed as sound on superficial examination even when the scales are removed, as the interior of the corm may to some extent be attacked without any external symptoms. Infected corms put out a shoot which sooner or later becomes attacked by the fungus growing out from the corm, and causing the foliage to turn yellow and wither away. Sclerotia are often produced at the collar region of the plant and Wade (1945) has described this as the collar-rot stage of the disease.

In addition to the above mode of attack young plants arising from clean corms are sometimes infected from the soil and show a failure of growth after the shoot has elongated a few inches. In these plants, sclerotia and mycelium are generally to be found in the collar region and isolations give a species of *Botrytis* as the causal agent. During the last two years losses due to such early attack have arisen mainly from the planting of diseased corms. Infection of healthy corms has been comparatively rare, probably because fresh ground has been used for planting.

(b) *Symptoms on flowers.* When the flowering period is well advanced, and especially if some of the flower stalks have not been cut early enough, first the older flowers at the base of the stalk, and later the younger ones, become profusely covered with conidia and, if the weather is humid, this is followed by strong development of aerial mycelium. In the red-flowered variety 'Van Tienhoven' and even in such a pale-coloured variety as 'Lilac Wonder' initial attack in the petals may be apparent as pale white or water-soaked spots or areas. The abundant conidial production on flowers is interesting because the species of *Botrytis* responsible for *Botrytis* rot of gladiolus is peculiar as it does not normally produce conidia readily on artificial media. Wade (1945) made the sound observation that flowers

left in the field contribute most of the spore load of this species of *Botrytis*, apart from causing direct loss by destruction of blooms.

(c) *Symptoms on leaves*. Leaves surrounding infected flowers may show numerous minute brown spots with definite reddish margins, or the spots may coalesce into larger blotches. From the latter the fungus is readily isolated, but from the former only occasionally; in this respect my experience agrees with that of Moore (1939) and of Wade (1945).

(d) *Symptoms on corms*. Moore (1939) was the first to recognize three forms of symptoms on the corms, as follows:

(1) Sunken rounded lesions, usually with a small cavity under the tightly stretched skin of the corm, a type of rot frequent in Canada but rarely seen in England. This is an arrested type of lesion, and there are good grounds for believing that English conditions are usually not warm enough to produce this localization of attack.

(2) 'Core-rot', commonly met with in England, leading to rotting and often to disappearance of the central tissue of the corm, which may thus appear as a ring.

(3) 'Spongy rot', affecting the whole tissue of the corm. This type of rot has been the commonest of all in my experience and every intergrade between this and core-rot has been seen. In advanced stages and under humid conditions, there is profuse mycelial development over the surface, and later the formation of large black coraloid masses of sclerotia. Small groups of conidiophores also occur on the sclerotia or on the general mycelium. The affected corms finally may become mummified.

#### ISOLATIONS

These were made from corms, leaves, flowers, and flower stalks of gladiolus attacked by *Botrytis* and gave the data which are collected in Table 1. Occasionally the isolates of *Botrytis* were accompanied by other fungi, e.g. *Penicillium*; the latter were disregarded. When, however, no *Botrytis* appeared, the other fungus was recorded, as shown in the last column of Table 1.

Table 1

Source of isolate	Description	No. of isolates	<i>Botrytis</i>		Other fungi
			Mycelial type	Conidial type	
Corms	Showing spongy rot	32	24	4	2 <i>Penicillium</i> 2 <i>Fusarium</i>
	Showing core and spongy rot	6	6	0	—
	Showing core rot	20	20	0	—
	Lesions on surface	5	4	0	1 <i>Fusarium</i>
Flowers	Covered with conidia	25	19	6	—
Leaves	Leaf blotches and specks	10	6	4	—
Flower stalks	Diseased vascular bundles	25	20	5	—
	from inside				
		123	99	19	5

Table 1 shows that a mycelial type of *Botrytis* was consistently isolated from various diseased parts of the plant. These mycelial isolates were similar in their general habit of growth and, as will be shown later, belong

to *B. gladiolorum* Timm. (1942), and they will be referred to as such. Isolates of *B. cinerea* were also obtained but the proportion of *B. gladiolorum* to *B. cinerea* was very high, namely, 99 to 19. It was thus early recognized that the species of *Botrytis* associated with the *Botrytis* rot of gladiolus was different from the usual species *B. cinerea*. The morphology, physiology and identity of the causal organism will be dealt with in a later part of this paper. No experiments have been carried out with the species of *Penicillium* or *Fusarium* which are noted in the last column of Table 1.

#### INOCULATION EXPERIMENTS ON GLADIOLUS

(a) *Corms*. Isolates of *Botrytis cinerea* from gladiolus and of *B. gladiolorum* were inoculated into corms which had been wounded at side, apex or base. Under the cool or cold conditions of winter storage the proportion of successful infections with *B. gladiolorum* was moderate: twenty-nine out of forty-four in one experiment and twenty-seven out of forty-one in another. Only seldom, for example in eight out of fifty-three samples, were unwounded corms infected. Under the same conditions inoculations with isolates of *B. cinerea* invariably failed. Moore and other workers have recorded similar experiences.

Temperature has a marked controlling effect on attack, as is shown by the data of Table 2.

Table 2

Temperature (° C.)	No. inoculated	No. infected
5-10	20	10 (slightly)
10-20	41	18
15	24	21
20-25	31	3

Inoculations at 15° C. consistently gave a high proportion of successful infections, and this result has been confirmed by the following experiment. Disks of gladiolus corm were inoculated at the centre with a small piece of mycelium of *B. gladiolorum* and stored under moist sterile conditions at temperatures of 5-10, 15, 20, 25 and 30°. Weights (g.) of rotted tissue produced in four days were as follows:

Temperature (° C.)	Weight (g.)
5-10	0.9
15	1.1
20	0.3
25	0.15
30	0

The greatest weight of rot produced was at 15° with a sharp drop as the temperature rose. On the other hand, the optimum for linear growth of the fungus was found to be about 22°, i.e. at a point where rotting of tissue is very slight. This is because gladiolus tissue readily forms cork at higher temperatures, as had been observed in the inoculation experiments already mentioned. At about 15° C. cork formation is sufficiently slow to enable the fungus to advance and hence the observed optimum for the development

of the rot. Below 15° C., although cork formation is weak, the growth of the fungus is also retarded, and this accounts for the slight spread of the rot in this temperature range which is apparent for example in Table 2.

It is not unlikely that the optimum temperature for attack may be slightly different in different varieties according to their cork-forming capacities. The temperature of 15° C. was optimum for 'Lilac Wonder', but no other varieties were tried. It is therefore probable that varietal susceptibility to storage rot is at least partly determined by periderm-forming tendencies.

The presence of a low optimum for development of storage rot agrees with the general observation during this investigation that storage rot is essentially a low temperature disease. Drayton (1929) also observed that a cool temperature is favourable for the decay of corms. This view is supported by the good control of the disease obtained by high temperature storage to be referred to later.

(b) *Flowers*. Isolations from diseased flowers yielded a much higher proportion of *B. gladiolorum* than of *B. cinerea*. A series of inoculation experiments with flowers in moist chambers gave the results summarized in Table 3. The spores were applied in water either in the form of a fine spray or in rain-drop size.

Table 3

Isolate	No. inoculated	No. infected
<i>B. gladiolorum</i> (from naturally infected flowers)	33 18	32 18
<i>B. cinerea</i> Isolate I	33	9
Isolate II	33	5

These results indicate clearly the greater pathogenicity of *B. gladiolorum*.

(c) *Leaves and shoots*. Table 4 gives the results of an experiment in which pieces of healthy leaves, after surface sterilization, were inoculated without wounding in a moist container.

Table 4

Isolate	No. of inoculations	No. of infections after			
		24 hr.	48 hr.	72 hr.	96 hr.
<i>B. gladiolorum</i>	20	4	4 definite, 4 slight	11 definite	12 definite, 4 slight
<i>B. cinerea</i> Isolate I	20	0	0	0	1 slight
Isolate II	20	0	0	2 slight	2 definite, 1 slight
Control	20	0	0	0	0

The effect of *B. gladiolorum* was apparent sooner, and the total number of infections was greater than with isolates of *B. cinerea*. The mycelium of *B. gladiolorum* was observed to penetrate right through the leaf to the other side. Rusty specks appeared at the margin of the lesion and were evidently caused at points of penetration of hyphae at an incipient stage of attack. These were very characteristic of *B. gladiolorum* and to a much less extent of the other strains.

In another experiment young gladiolus plants about 1 ft. tall, grown in moist sand, were inoculated on the leaves from agar cultures of



*B. gladiolorum* and *B. cinerea*. High humidity was maintained by covering the plants with bell jars. Here again rusty specks developed which coalesced into spreading lesions in the inoculations with *B. gladiolorum*. *B. cinerea* showed only a comparatively low degree of virulence (Table 5).

Table 5

Isolate	No. of plants	No. of leaves inoculated	Attack after 1 week	Attack after 2 weeks
<i>B. gladiolorum</i>	10	14	10	14
<i>B. cinerea</i> Isolate I	10	15	0	2
Control (agar only)	10	15	0	0

Somewhat more normal conditions were obtained in an experiment in which corms were allowed to grow in pots of sterilized soil in the greenhouse until almost the stage of flowering. Two healthy corms were planted in each of ten pots and at the time of inoculation the plants were nearly 30 in. tall. The inoculum, which consisted of a piece of an agar culture of *B. gladiolorum*, was placed in the axil of the second leaf above the soil. Sixteen shoots arising in five pots were thus inoculated, while fifteen shoots in the remaining five pots were used as controls. All the pots were placed in a humidity chamber for five days after inoculation, and then returned to the open air of the greenhouse.

Within a week, water-soaked lesions developed on some of the shoots of the inoculated series, and at the end of a fortnight every inoculated shoot showed spreading lesions which mostly developed downwards and round the shoot. The controls meanwhile remained healthy. In three weeks fourteen out of sixteen shoots of the inoculated series were so rotted that the tops generally buckled over at the point of inoculation. At the end of a month the rot had spread to the corms, which became soft, and typical conidiophores of *Botrytis* were seen at the collar region and on the apex of the corms.

Similar inoculations were carried out under field conditions. In September 1945, three types of inoculum were used, viz. *B. gladiolorum*, *B. cinerea* (eight-day-old cultures of each) and a one-month-old culture of the latter. Plants were inoculated one month before lifting, in the leaf axil just above ground level as in the above experiment, and controls were inoculated with sterile agar. Seventy-two shoots were used for each treatment. Table 6 shows the results.

Table 6

Isolate	No. of shoots visibly diseased after 10 days	Losses during growth	Percentage of corms rotted in store
<i>B. gladiolorum</i> (8-day-old culture)	52	2 <i>Botrytis</i>	58.3
<i>B. cinerea</i> (8-day-old culture)	4	1 <i>Botrytis</i>	51.4
<i>B. cinerea</i> (1-month-old culture)	1	3 <i>Sclerotinia</i>	44.9
Control (agar only)	0	1 <i>Botrytis</i>	48.6

Table 6 shows that within ten days a high proportion of the plants (viz. fifty-two out of seventy-two) inoculated with *B. gladiolorum* developed

discoloured lesions at the point of inoculation, while the figures were four out of seventy-two for the young and one out of seventy-two for the old culture of *B. cinerea*. However, this high proportion of initial attack was not reflected in disease incidence in subsequent storage. The loss in storage of the lot inoculated with *B. gladiolorum* was not appreciably greater than of those inoculated with *B. cinerea* or of the controls.

In September 1946 a similar series of inoculations was made in the leaf axils with *B. gladiolorum* and with two isolates of *B. cinerea*. The percentage of successful inoculations was neither so great nor were the lesions so distinct as in the previous year, probably due to the lack of favourable weather conditions at the time. The corms were lifted a month afterwards, cleaned and stored in the usual manner and examined for *Botrytis* rot at the end of ten weeks (Table 7).

Table 7

Isolate	No. of shoots inoculated	No. of new corms	No. rotted 10 weeks after lifting	Total no. rotted in storage	Percentage of rotted corms
<i>B. gladiolorum</i>	60	60	4	54	90
<i>B. cinerea</i> Isolate I	60	60	2	40	67
<i>B. cinerea</i> Isolate II	60	60	0	39	65
Control (agar only)	60	60	2	45	75

Here again the increased incidence of corm rot arising from inoculating the shoots with *B. gladiolorum* was slight.

Similar results were obtained where inoculations were made, in September 1946, at the cut ends of the flowering shoots. Five weeks before lifting, the plants were cut 4-5 in. from the ground and inoculated with the same isolates as in the previous experiment, drops of sterile water being added occasionally to keep the inoculum moist. The resulting number of corms rotting in storage ten weeks after lifting and at the end of the storage period are set out in Table 8.

Table 8

Isolate	No. of shoots inoculated	No. of new corms	No. rotted 10 weeks after lifting	Total no. rotted in storage	Percentage of rotted corms
<i>B. gladiolorum</i>	25	25	3	25	100
<i>B. cinerea</i> Isolate I	25	25	1	24	96
<i>B. cinerea</i> Isolate II	25	25	2	22	88
Control (agar only)	40	40	0	35	87

It is probable that in the field experiments mentioned above, ideal conditions for infection or further spread of the fungus or for both did not prevail. This is particularly noticeable in the experiments for 1945 and 1946 where axils of plants were inoculated (Tables 6 and 7). Whereas in 1945 the parts above ground were strongly attacked but advance into the corm was poor, in 1946 even foliage infection was very slight. A study of rainfall figures shows that though the total precipitation for the fortnight following inoculation in the two years was not quantitatively different, it was more evenly distributed in 1945. The possibility of loss in vigour of the isolate of *B. gladiolorum* can be eliminated because greenhouse experi-

ments carried out at about the same period as the above experiments were very successful.

As far as is known there are no other reports of inoculations of gladiolus plants in the field at a post-flowering stage with *Botrytis*. Wade (1945), however, obtained typical *Botrytis* rot in corms that were inoculated at the cut ends after digging and enclosed in tins, but none in those inoculated and stored on a wire-netting stretcher. It is also interesting in this connexion to note that although he produced heavy infection of leaves by brushing them with conidia, no *Botrytis* rot subsequently developed in storage.

Timmermans (1941) considered that infection travels along the vascular bundles into the corms, and suggested that when the latter could not be stored at a high temperature the stalks should be torn off immediately after lifting and not cut off at about 1 in. distance, as is the usual practice. An experiment carried out in the autumn of 1946 to test the value of this method did not give promising results, and in fact there was a higher percentage of losses in the batch treated according to Wade's suggestion. Thus, except possibly under special circumstances, as for example when heavy foliage infection occurs, there is no advantage in breaking off the tops of corms immediately after lifting. On the contrary, this might expose the corms to easier invasion by *Botrytis* and other fungi through the uncalled wound on the corm thus left exposed. It is therefore reasonable to conclude that infection occurs to a large extent either through the cut end or through the scar at the apex. Basal infection through the old corm is also evident though this, according to observations, is not as frequent as that from the apex. Natural infection through the basal scar after cleaning is to some extent another point of entry.

The following general conclusions are to be drawn from the preceding experiments:

(1) *B. gladiolorum* is capable of quickly setting up infection on growing gladiolus plants and, under favourable controlled conditions such as are obtained in a greenhouse, travels down to the corm and sets up rot in it.

(2) In the fluctuating environment of the field *B. gladiolorum* is much more pathogenic than isolates of *B. cinerea*. Although plants inoculated with *B. gladiolorum* gave a higher number of diseased corms in storage than did the controls and those inoculated with *B. cinerea*, this difference, however, was small. A high proportion of foliage infection in the field was not associated with a correspondingly high disease incidence in storage. This is probably because favourable conditions for infection and spread of the fungus in the field were not realized during these experiments.

(3) Irrespective of the infection of the shoot and transmission of the disease through the shoot into the corm, much rot always occurred in storage. The obvious interpretation is that this is due to natural infection of corms after lifting, either through the cut end of the flowering stalk after trimming or through the stem scars left at the top and bottom of the corm at cleaning. The leaf scars which occur round the corms are probably of little importance, inasmuch as side lesions are far less frequent.

EFFECT OF CULTURAL AND STORAGE CONDITIONS ON  
ROTTING OF CORMS

The experiments in this connexion follow the lines of earlier work by Hawker (1946) and substantially confirm her findings.

A number of rows of plants growing in the field were protected from wetting and a similar number were watered in addition to receiving the natural rainfall. These treatments were maintained for the three weeks preceding lifting. The percentage losses from *Botrytis* in the stored corms were 24 and 47 % in the batches from dry and wet soil respectively.

The effect of dates of lifting and cleaning is shown in Table 9.

Table 9

Date of lifting	Condition of soil at lifting	Date of cleaning	Percentage rotted
12 October	Dry	1 November	12
6 November	Very wet	20 November	16.4
22 November	Very wet	12 December	64.2
12 October	Dry	10 January	54.1
6 November	Very wet	10 January	95.4
22 November	Very wet	10 January	90

It is evident from Table 9 that when the plants are lifted early, storage losses are low, while the later they are left in the ground the more disease in storage increases. This is probably due to longer exposure to infection, to the greater tendency for the ground to get wet as the season advances, and to the slower rate of drying of the later lifted corms owing to the comparatively low temperatures prevailing at lifting.

The effect of conditions during the early period of storage is shown in Table 10.

Table 10

Conditions of storage during first month	Temperature (° C.)	Percentage diseased	
		<i>Botrytis</i>	Other fungi
Cool and moist	12-15	70	None
Cool and dry	12-15	46	6 <i>Septoria</i> , 2 <i>Sclerotinia</i>
Warm and moist	17-20	20	2 <i>Sclerotinia</i> , 4 <i>Penicillium</i> and <i>Sclerotinia</i> , 6 <i>Septoria</i>
Warm and dry	17-22	8	18 <i>Sclerotinia</i> , 4 <i>Penicillium</i>

It is clear from Table 10 that storage for one month at high temperature was very effective in the control of *Botrytis* disease. Warm dry conditions were better than warm moist conditions, emphasizing the necessity of good ventilation as well. The greatest number of diseased corms was obtained under cool moist conditions, and this agrees with the results of the inoculation experiments mentioned earlier, which showed that low temperatures favoured the disease. On the other hand, it is likely that other fungal troubles were increased by the warmer storage conditions, as shown in the last column of Table 10. From a similar experiment, Hawker (1946) concluded that the control of the disease by this means was due to the

increased rate of drying of corms and the formation of callus over wounds. Timmermans (1941) also emphasized that storing immediately after lifting at 25–30° C. gives good control of *Botrytis* rot.

#### INOCULATION EXPERIMENTS WITH *BOTRYTIS GLADIOLORUM* AND STRAINS OF *BOTRYTIS CINEREA* ON OTHER HOSTS

In this work, four isolates of *B. cinerea* were used:

Isolate I. A conidial strain from gladiolus (GI).

II. A conidial-sclerotial strain from gladiolus (GII).

III. A conidial-sclerotial strain from lettuce (L).

IV. A conidial-sclerotial strain from daffodil leaves (D).

Inoculations were made in a standardized manner into roots of swede and turnip, and on lettuce leaves; also, for comparison, into gladiolus corms and on gladiolus leaves. With swede, turnip and gladiolus corms, the amount of rotted tissue was weighed, and as the inoculations were replicated, results were obtained which could be treated statistically. The results are summarized in Table 11.

Table 11

Host	Fungus	Replications	Weight of rot (g.)
Turnip	<i>B. gladiolorum</i>	10	0.04
	<i>B. cinerea</i> (G I)	10	0.27
	(G II)	10	1.63
	(L)	10	2.11
	(D)	10	0.67
Swede	<i>B. gladiolorum</i>	6	0.01
	<i>B. cinerea</i> (G I)	6	2.91
	(G II)	6	3.71
	(L)	6	4.52
	(D)	6	3.00
Gladiolus	<i>B. gladiolorum</i>	7	0.89
	<i>B. cinerea</i> (G I)	7	0.14
	(G II)	7	0.11
	(L)	7	0.13
	(D)	7	0.13

The differences in amount of attack between *B. gladiolorum* and any of the *B. cinerea* strains are highly significant on all three hosts, but whereas *B. gladiolorum* is the active fungus on gladiolus corms, it is much the weakest on the other two hosts, and conversely for the four strains of *B. cinerea*. The results with lettuce and gladiolus leaves, though less susceptible of quantitative treatment, were of a similar type. There is thus a clear distinction in pathogenic behaviour between *B. gladiolorum* and four isolates, chosen more or less at random, of *B. cinerea*.

#### CONIDIAL PRODUCTION OF *BOTRYTIS GLADIOLORUM*

In a comprehensive series of experiments (not included here) on the effect of cultural and environmental factors on the growth characters of *B. cinerea* (several strains) and *B. gladiolorum* it was found that the latter consistently produced very few conidia. This observation agrees with

those of Wade (1945) and Dodge and Laskaris (1941) who found that the isolate of *Botrytis* obtained by them as the causal agent of *Botrytis* rot sporulated weakly in culture; also with Timmermans's (1942) finding that *B. gladiolorum* is a poor conidial producer in culture. Nevertheless, it was shown above that conidia are formed freely on flowers and to a less extent on flower shoots, leaves and corms of gladiolus. Formation of conidia in nature is obviously essential if the view is to be maintained that the predominant species of *Botrytis* in causing gladiolus *Botrytis* rot is *B. gladiolorum*. Special attention was therefore given to this subject.

Illumination of fairly strong intensity appears to be required for the sporulation of *B. gladiolorum*. Thus spores were freely produced on fresh flowers in Petri dishes when kept in front of the laboratory window in summer time, but not when kept in the dark.

Petri dishes containing a glucose-peptone medium (glucose, 0.5 %; peptone, 0.6 %, with mineral salts) were inoculated and placed (a) in front of a window under direct illumination for about four hours a day, (b) in diffused light, and (c) in the dark. At the end of four days normal erect grey conidiophores developed in the dishes in direct light but not in the other two sets. Under the conditions of (a) there is considerable condensation of moisture on the lids of the Petri dishes, and this must have been extracted from the culture medium, thus leading to a certain degree of desiccation. The latter factor in itself tends to increase sporulation of many fungi, including *B. cinerea* and *B. gladiolorum* (see below), but subsidiary experiments showed that light was the essential factor. Thus conidial formation was not materially affected by turning the dishes upside-down (which eliminates condensation on the lids), and conversely no conidia were produced in blackened Petri dishes placed with the lids uppermost, though here condensation of moisture was well marked.

Of the media tested, the glucose-peptone medium and an extract of gladiolus leaves proved to be the most suitable. Potato and malt extracts were of little value.

That a certain degree of drying accentuates spore production has been shown by Reidmeister (1909), Wade (1945) and others. The following method, which combines the effects of light, suitable medium, and a certain amount of drying, was developed and proved satisfactory for the supply of spores of *B. gladiolorum* for experimental purposes. The fungus was grown in the light on the liquid glucose-peptone medium already mentioned, and after ten to fourteen days the hyphal mats were placed on sterile filter-papers on wire gauze in large dry glass dishes. These were then kept exposed to light. Within two to three days numerous conidia developed on the slowly drying mats, especially round the edge and on the originally lower surface.

A point of interest that appeared in these experiments was that the conidia of *B. gladiolorum* germinate comparatively easily. Dishes which showed an abundance of conidia would, especially under favourable humidity, show a replacement of the conidia with white woolly mycelium within four or five days. This tendency was not apparent in *B. cinerea* where conidia persisted as such for a long time.

DESCRIPTION OF *BOTRYTIS GLADIOLORUM* TIMMERMANS, THE  
CAUSAL ORGANISM OF *BOTRYTIS* ROT OF *GLADIOLUS*

This fungus is sufficiently distinctive in its cultural and morphological features to merit specific status. In its pathogenic behaviour it stands in strong contrast to the other isolates of *Botrytis* tested in that it was the only one which actively invaded gladiolus tissues; conversely it stood out from the remainder in causing negligible attack on such hosts as turnip, swede and lettuce, on which isolates of *B. cinerea* were active.

The following description of the fungus is based on my experience, and is in substantial agreement with Timmermans's account.

*Mycelium* on gladiolus corms white woolly, often profuse; on artificial media similar; mature hyphae 8–12  $\mu$  diameter, finer branches down to 3  $\mu$ ; in culture grey-green appressoria formed along the glass of Petri dish or tube, but this feature tends to become less prominent in long-established cultures.

*Sclerotia* on corms developed abundantly, first as smooth creamy white bodies soon becoming black and by-and-by usually coalescing into large coralloid masses; in artificial cultures usually occurring rarely and then in old cultures (one month old). (In contrast to this, sclerotium-forming isolates of *B. cinerea* formed sclerotia in comparatively young cultures). Sclerotia normally small in culture, but reaching 1 cm. in length on a glucose-peptone medium; in later stages of the work showing a tendency to become submerged in the medium.

*Microconidia* formed on most media on small side branches of the hyphae, small, globose, 2  $\mu$  in diameter.

*Macroconidia* freely produced on flowers in the field and to a less extent on corms; in artificial culture sparingly formed and tending to become rarer by continued subculturing; produced more freely under conditions of abundant light, etc., as outlined in the preceding section; borne on simple or branched conidiophores which are greyish brown (not dark brown as described for *B. gladioli* by Klebahn), but the precise colour is probably conditioned by the environment, as darker conidiophores have been seen in culture on glucose-peptone medium in good light; conidiophores paler towards top, average diameter up to 18  $\mu$ , cells variable in length; conidia borne on ampullae on main stalk or side branches, terminal or lateral; uniform in size, averaging 13  $\times$  8  $\mu$ , generally 1-celled, ovoid, formed in the presence of light, germinating generally by one or sometimes two germ tubes.

In addition to the normal conidia, abnormal conidia are often seen; these arise on more hyaline conidiophores. The end of such a hypha swells to varying degrees and buds off the conidia terminally or laterally. Often the whole ampulle with its buds is detached or the end of a hypha forms a spore without budding. These spores are sometimes bicellular, but their most striking feature is their variable length, which may reach 50  $\mu$ . They may sometimes be found germinating in situ. They are generally found in cultures which have received suboptimal illumination, but may also occur

mixed with conidia of the ordinary type. Similar structures were seen in a culture of *B. gladiolorum* supplied by Dr Timmermans.

After the conidia have been formed the ampullae always shrivel in the way figured by Timmermans (1942).

Average spore measurements from my data and from the literature are assembled in Table 12.

Table 12

Author	Isolate	Av. spore measurement	Quotient*
Peiris	<i>B. gladiolorum</i>	13.6 × 10.2	1.33
	<i>B. cinerea</i>	10.1 × 8.0	1.24
Timmermans (1942)	<i>B. gladiolorum</i>	15.0 × 10.0	1.5
Moore (1939)	<i>Botrytis</i> from gladiolus	13.0 × 10.0	1.3
Wade (1945)	<i>Botrytis</i> from gladiolus	15.0 × 12.0	1.25
Dodge & Laskaris (1941)	<i>Botrytis</i> from gladiolus	15.8 × 10.5	1.5
Klebahn (1930)	<i>B. gladioli</i>	10.4 × 4.7	2.21

\* Quotient = length ÷ breadth

It is evident that with the exception of *B. gladioli*, the other isolates from gladiolus approximate to the *B. gladiolorum* of Timmermans. The spores of *B. gladioli* were measured in glycerine, and Klebahn suggests that for comparison the readings should be increased by one-third. Even after making this allowance, the average measurements for *B. gladioli* would be  $13.8 \times 6.3 \mu$ , the spores being characterized by their narrowness as reflected by the high quotient 2.21. The other nine species of *Botrytis* which Klebahn studied had a markedly smaller quotient. These measurements and the peculiar jagged or toothed sterigmata of *B. gladioli* have not been observed by subsequent workers, and it is unknown whether *B. gladioli* was pathogenic on gladiolus, as Klebahn did no inoculation experiments. Wade (1945) is doubtful whether his isolate, in spite of the difference in spore measurements, could be regarded as a different species from *B. gladioli*. There are, however, strong resemblances between Wade's isolate and that described by Timmermans (1942) and here as *B. gladiolorum*. The fungus described by Dodge and Laskaris (1941), though not worked out in detail, also shows similarity with *B. gladiolorum* especially in its poor sporing capacity in culture. The isolates of Moore (1939) and Hawker (1946) were recognized by them to be different from *B. gladioli*. It is possible that they were isolates of the species *B. gladiolorum*.

#### SUMMARY

1. The symptoms of the disease studied are in substantial agreement with those described by Moore and the three types of corm symptoms mentioned by him are different phases of the same disease.

2. The symptoms are constantly associated with a species of *Botrytis* different from *B. cinerea*. Morphological and cultural examination of this fungus shows that it is *B. gladiolorum* Timm.

3. Inoculation experiments through wounds on corms showed that *B. gladiolorum* is more pathogenic on gladiolus than *B. cinerea*. The rate of attack is greatest at a storage temperature of about 15° C.

4. On flowers and shoots *B. gladiolorum* is also more pathogenic, but although under ideal conditions foliage attack caused subsequent corm



rot, this is probably not of major importance in regard to storage rot. The evidence shows that the higher proportion of infection leading to storage rot occurs after lifting the corms, probably on the drying racks.

5. A useful measure of control of the disease is obtained by avoiding planting in heavy soil liable to waterlogging, by early lifting, by rapid drying at a high temperature, and by early cleaning.

6. *B. gladiolorum* was pathogenic on gladiolus whereas the isolates of *B. cinerea* were comparatively inert. Converse pathogenicity was observed when turnips, swedes and lettuce were used as hosts.

7. *B. gladiolorum* tends to be sterile in artificial culture. Media suitable for sporulation are a decoction of gladiolus leaves and glucose-peptone. Formation of conidia occurs only in good light and is also accentuated by moderate drying of the culture medium.

8. A description of *B. gladiolorum* is included which is in agreement with that of Timmermans. The features of this isolate are considered to be sufficiently distinctive to entitle it to specific status. The isolates of *Botrytis* from gladiolus made by earlier workers except Klebahn show in greater or less degree similarities with *B. gladiolorum* and were probably isolates of *B. gladiolorum* Timmermans.

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# SPONTANEOUS MUTATION IN STANDARD AND 'GIGAS' FORMS OF *PENICILLIUM NOTATUM* STRAIN 1249 B21

By EVA R. SANSOME

*Department of Cryptogamic Botany, University of Manchester*

(With Plate XXIII and 1 Text-figure)

Camphor-induced 'gigas' forms have been produced by Bauch in yeast and other fungi (Bauch, 1941*a, b*), in *Torulopsis utilis* by Thaysen and Morris (1943), in *Saccharomyces cerevisiae* (Subramaniam, 1945) and in *Penicillium* (Sansome, 1946; Kostoff, 1946). Bauch stated that natural camphor was more active than synthetic camphor. The sample of camphor used in these experiments was determined by Prof. Brindle to be dextrorotatory and it is, therefore, most probably natural camphor. Skovsted (1947), using synthetic camphor, was unable to obtain diploid from haploid strains of *Saccharomyces cerevisiae*. Kostoff obtained *gigas* forms after acenaphthene treatment as well as after treatment with camphor.

What is the nature of these *gigas* strains? Bauch assumed them to be diploids, presumably on the basis of the size difference which is of the order to be expected if the original forms are haploid and the *gigas* forms are diploid. The cytology of these forms is very difficult, and so far no convincing direct cytological evidence regarding chromosome numbers in these forms has been obtained. In *Penicillium notatum* strain 1249 B21, certain mutant types occur with a high frequency, as previously described (Sansome, 1947). It was thought that if *gigas* types of this strain could be obtained, a study of mutation in the standard and *gigas* forms might provide genetical evidence as to the nature of the *gigas* forms.

## EXPERIMENTAL

Plates of malt-extract agar were prepared with small amounts of camphor and inoculated together with control plates without camphor. The cultures could not be placed in the incubator because of the risk of treating other cultures in the incubator with camphor vapour. They were, therefore, left in the laboratory under bell jars at a much lower temperature than that of the incubator. The necessity of growing the cultures at a much lower temperature than usual may have been an advantage, since Levan (1947) has shown that the action of camphor and other substances on the growth of yeast is intensified at lower temperatures. Camphor has a powerful inhibitory effect on the growth of *Penicillium*. The hyphae are short, much branched and twisted, forming a small colony with an irregular wavy edge. Conidial formation is much delayed and it is possible that it occurs only after the concentration of camphor has been reduced by evaporation.

When conidia appeared they were plated out by the dilution method and a number of single conidial cultures were isolated into tubes of malt-extract agar. These were later examined for conidial size. No cultures were found to have distinctly larger conidia than the controls, but there was much variation in conidial size which made the recognition of *gigas* cultures difficult. The experiment was, therefore, repeated, using glycerine-molasses-peptone agar which induces heavy conidial formation; since it was thought that this medium would also lead to the production of conidia more regular in size. This occurred, and as a result of the second experiment two out of thirty cultures were obtained, with conidia larger than those of the controls. It must also be mentioned that one out of thirty of the controls had larger conidia also. From this experiment it appears *gigas* forms may appear spontaneously in this strain when growing at rather low temperatures and it is not certain that the two *gigas* forms obtained in the treated series were actually the result of the treatment.

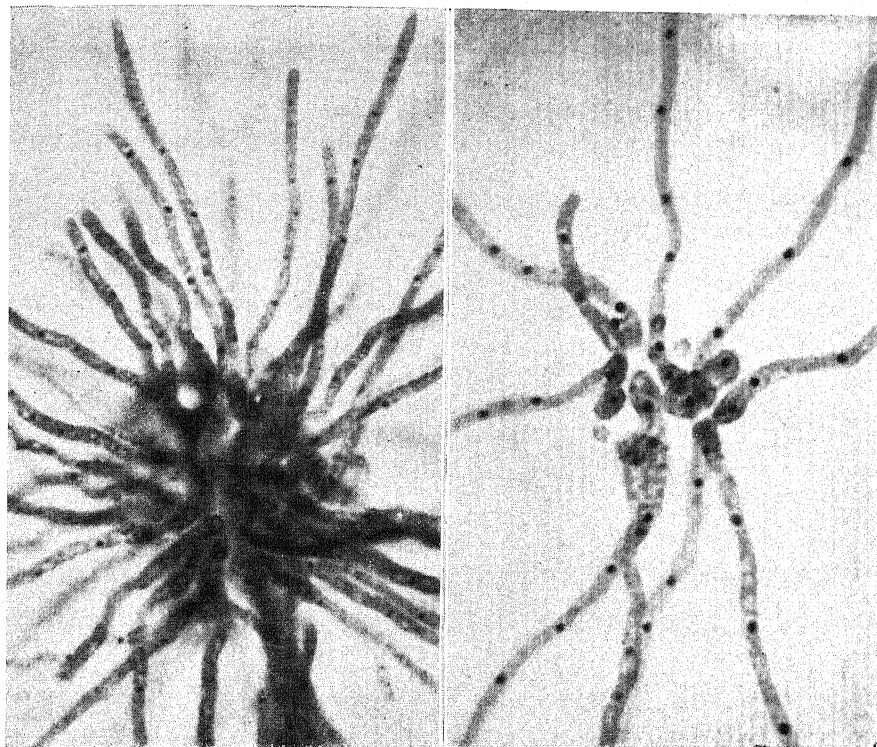
#### *Comparison of standard and gigas forms*

The average diameter of the conidia of the *gigas* form is  $4.2\mu \pm 0.04$  as compared with  $3.0\mu \pm 0.01$  for the standard. The ratio is thus 1.4:1. The probability  $p$  that the two types of conidia belong to one group is less than 0.01. The penicilli of the *gigas* form are slightly larger than those of the standard as illustrated in Text-fig. 1. There is much variability in hyphal width so that comparisons between the two types for this characteristic are difficult. Pl. XXIII shows germinating conidia of *gigas* and standard forms of strain 1249 B21 fixed in formol alcohol and stained with Heidenhain's haematoxylin. The size difference, less easy to detect in the ungerminated conidia is obvious in the swollen germinated conidia. A point of special interest is that the nuclei of the *gigas* form are much larger than those of the standard type. This is in accordance with the hypothesis that the *gigas* type is diploid although not, of course, a proof of it, since a general size factor would affect nuclear as well as cell size.

Apart from the microscopic size differences the *gigas* and standard forms are identical in appearance and when inoculated together they give a colony completely homogeneous in appearance.

#### *Mutation in gigas and standard forms*

Table 1 shows the spontaneous mutant colonies obtained by plating out eight single conidial cultures of the standard type. There were 0.7% of *dark* 1 variants, 0.9% of *pale* 1 variants and 0.2% of other variants. Three-quarters of the last class came from one culture and consisted of similar mutants which probably came from one original mutation. Every culture gave both *pale* 1 and *dark* 1 mutants but one, in which the *pale* 1 type was missing. In this case only 281 colonies were recorded, and it is possible that if more had been examined the *pale* 1 type would have been found here also. Of the total mutants amounting to 1.7% over 1.5% were either *pale* 1 or *dark* 1. This is in accordance with previous results, in which it was

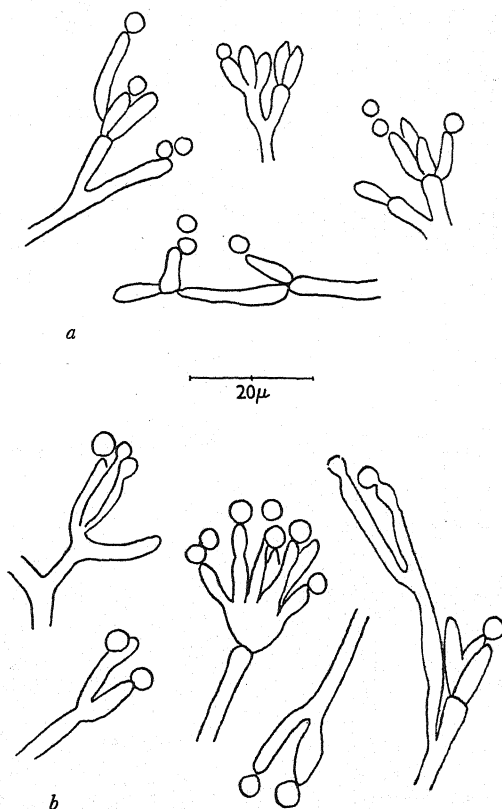


*a*

*b*

Germinated conidia of (*a*) standard, (*b*) *gigas* strains, fixed formol-alcohol, stained with Heidenhain's haematoxylin,  $\times 1000$ .





Text-fig. 1. Penicilli from (a) standard, (b) *gigas* forms. The metulae and sterigmata are longer in the *gigas*-form.

Table 1. *Spontaneous mutants in control standard*

Age of single conidial cultures (days)	Total colonies recorded	Dark 1	Dark 1 (%)	Pale 1	Pale 1 (%)	Others	Others (%)	Total	Total (%)
11	1345	6	0.45	19	1.41	1	0.17	26	1.93
11	532	9	1.69	9	1.69	—	—	18	3.38
11	1338	4	0.30	7	0.52	—	—	11	0.82
14	932	9	0.97	15	1.61	—	—	24	2.58
99	225	5	2.22	1	0.40	—	—	6	2.67
11	541	2	0.37	2	0.37	9	1.66	13	2.40
11	1247	5	0.40	3	0.24	—	—	8	0.64
14	281	4	1.42	—	—	2	0.71	6	2.14
Total	6441	44	0.7	56	0.9	12	0.19	112	1.74

shown that the greatest part of the variability of strain 1249 B21 is due to the occurrence of these two types of mutants (Sansome, 1947).

Table 2 shows the spontaneous mutants from thirteen single conidial cultures of the *gigas* form. The cultures marked with an asterisk had small

darker sectors. The remainder were uniform in macroscopic appearance. Every culture gave a certain number of paler and darker variants. The total number of variant colonies was 226 out of 4872 or 4.6%; excluding the sectored cultures the variants were 4.3%. The darker variants amounted to 2.5% and the paler variants to 1.1% when the sectored cultures were included. When the sectored cultures were excluded the darker variants amounted to 2.0% and the paler variants to 1.2%. Spasmodically occurring mutants of different types amounted to 1.1% of the total and were thus more frequent in the *gigas* form than in the standard, where they were only 0.2% of the total colonies.

Table 2. *Spontaneous mutants in standard gigas form*

Age of single conidial cultures (days)	Total colonies recorded	Dark variants	Dark variants (%)	Pale variants	Pale variants (%)	Other variants	Other variants (%)	Total variants	Total variants (%)
11	592	6	1.01	3	0.51	—	—	9	1.52
11	631	14	2.22	2	0.32	—	—	16	2.54
11	240	6	2.50	5	2.08	1	0.42	12	5.00
11	273	2	0.73	4	1.47	1	0.37	7	2.56
11	412	6	1.46	2	0.49	5	1.21	13	3.16
11	185	1	0.54	1	0.54	—	—	2	1.08
14	353	7	1.98	4	1.13	3+7 sect.	2.83	21	5.95
	238	3	1.26	3	1.26	—	—	6	2.52
41	271	16	5.90	6	2.21	9	3.32	31	11.44
42	476	11	2.31	13	2.73	18	3.78	42	8.82
11*	372	23	6.18	2	0.54	1	0.27	26	6.99
11*	387	20	5.17	5	1.29	4	1.03	29	7.49
11	442	7	1.58	2	0.45	3	0.68	12	2.71
Total	4872	122	2.5	52	1.1	45+7 sect.	1.1	226	4.6
Excluding sectored cultures	3671	72	2.0	43	1.2	37+7 sect.	1.2	1159	4.33

The darker and paler types were more difficult to record in the *gigas* form than in the standard, being intermediate between standard and *dark* 1 and standard and *pale* 1 respectively. However, with practice it was possible to record the darker variants with reasonable accuracy. The recording of the pale types was more difficult and subject to greater error. A number of the dark variants coming from different cultures were inoculated together. They gave apparently homogeneous colonies.

Since the dark and pale variants in the *gigas* form were intermediate in appearance between the standard type and *dark* 1 and *pale* 1 respectively, it was thought that they might be heterozygotes for the *dark* 1 and *pale* 1 factors, one chromosome being changed, the other not. If so, the intermediate types might be expected to give mutants more closely resembling the mutants from the original standard type by the mutation of the second chromosome. The mutants would be expected to occur about as often as in the original standard type. The intermediate dark type was chosen for more detailed study rather than the intermediate pale for two reasons. In the first place, the intermediate darks were more easy to record;

secondly, the original dark variant was relatively stable whereas the *pale* 1 type was unstable. Consequently, the mutational pattern of the intermediate dark type might be expected to be simpler than that of the intermediate pale type. One or two of the intermediate pale cultures were plated out and they gave a range of paler variants in accordance with this expectation.

Table 3. *Spontaneous mutants in intermediate dark gigas form*

Age of single conidial cultures (days)	Total	Dark mutants	Dark mutants (%)	Paler mutants	Paler mutants (%)	Other mutants	Other mutants (%)	Total mutants	Total mutants (%)
14	1032	8	0.78	33	3.20	5	0.48 sect.	46	4.46
12	801	9	1.12	—	—	11	1.37 sect.	20	2.49
12	224	13	5.80	3	1.34	2	0.89	18	8.04
12	936	8	0.86	11	1.18	23	2.46 sect.	42	4.49
12	360	6	1.67	1	0.28	3	0.83	10	2.78
40	338	8	2.37	9	2.66	4	1.18	21	6.21
Total	3691	52	1.41	57	1.54	9+39 sect.	1.30	157	4.25

Table 3 gives the spontaneous variants from six single conidial cultures of the *gigas* intermediate dark type. Each culture gave a number of darker types, corresponding in appearance with the *dark* 1 type from the original 1249 B21. When such dark types of different origin were inoculated side-by-side they gave apparently homogeneous colonies. Similarly, when this *gigas* 2-step dark was inoculated with a standard 1-step dark an apparently homogeneous colony resulted. So far as may be tested, therefore, the *gigas* form gives, in two steps, the mutant given in one step by the standard form, as expected if the *gigas* form is diploid and the intermediate type is the heterozygote. This expectation is dependent upon the mutation being of nuclear rather than cytoplasmic origin.

The percentage of 2-step darks was 1.4, rather more than the 0.7% of *dark* 1 mutants produced by the standard and less than the 2.0% of intermediate dark mutants produced by the *gigas* type. These percentages are in the relationship to be expected, but too much weight must not be attached to this because of the great variance.

A point of interest is that although the original *dark* 1 type was very stable, giving only rare *dark* 2 variants, and never reverting to the parent type, the intermediate *gigas* darks gave some paler types. Some of these paler types were identical with the original *gigas* type so far as could be tested.

*The exceptional 'intermediate dark' from the standard 1249 B21*

In one of the control cultures plated at the same time as the *gigas* intermediate darks a variant strongly resembling the intermediate dark type appeared along with the usual *dark* 1 type. The possibilities that the control might have been a spontaneous *gigas* form or that the intermediate darks might be contaminants from the *gigas* cultures, were eliminated by



an examination of the conidial size. The exceptional variant and its sister colonies were found to have conidia of the standard size.

A number of single conidial cultures of this intermediate dark type were obtained and plated out. Table 4 gives the spontaneous mutants obtained from five of these cultures. There were 0.3% of darker mutants and 0.7% of paler mutants. However, these mutants were quite unlike those given by the *gigas* intermediate dark type, the dark types being much darker and the pale types much paler. Although this exceptional dark type closely resembles the *gigas* 1-step dark on the basis of our first criterion, appearance, it has a very different mutational pattern and must, therefore, be assumed to be genetically distinct. The simplest explanation is that it represents a different mutation which intensifies the effects of the *pale* 1 and *dark* 1 mutations.

Table 4. *Spontaneous mutants in intermediate dark from control*

Age of single conidial cultures (days)	Total colonies	Darker mutants	Darker mutants (%)	Paler mutants	Paler mutants (%)	Other mutants	Other mutants (%)	Total mutants	Total mutants (%)
15	490	—	—	—	—	2 sect.	0.41	2	0.41
28	580	2	0.34	3	0.52	—	—	5	0.86
21	159	2	1.26	2	1.26	—	—	4	2.52
21	357	1	0.28	5	1.40	—	—	6	1.68
21	206	1	0.49	3	1.46	—	—	4	1.94
Total	1792	6	0.33	13	0.72	2	0.11	21	1.17

#### DISCUSSION

*Gigas* forms of 1249 B21 have been shown to give a mutant type identical in appearance with the *dark* 1 type given by the parent form. However, they give the mutant in two steps, the first change being to a type intermediate between the standard and *dark* 1 types. These results are readily explicable if the original type is haploid and the *gigas* form diploid. In that case the intermediate dark would be the heterozygous dark formed by mutation of one chromosome to the *dark* 1 type. Mutation of the unchanged chromosome of the heterozygous type would give the type identical in appearance with the *dark* 1 of the original form.

If the proportion of nuclei mutating to *dark* 1 in the haploid is  $x$ , the proportion mutating to the intermediate dark type in the diploid would be  $2x - x^2$ , and the proportion of nuclei mutating directly to the *dark* 1 type would be  $x^2$ . Since  $x^2$  would be a small quantity, there should be almost twice as many nuclei mutating to the intermediate dark type in the diploid as to the *dark* 1 type in the haploid. Actually, there were 0.7% of *dark* 1 types in the control cultures and 2% of darker mutant colonies in the *gigas* cultures (excluding those cultures with a visible mutant sector). This is in accordance with expectation but the difference is not significant because of the great variance. Also, it must be emphasized that the proportion of mutants recovered depends upon other factors than the mutation rate: such as time of occurrence of mutations, relative survival values of original and

mutated types and degree of conidial formation of the two types. Furthermore, the Sewall-Wright effect which acts in favour of the nuclei present in the greatest numbers would be less strongly in favour of the original type when the number of mutations is increased. This probably accounts for the increase in colonies with visible mutant sectors in the *gigas* form.

The change from intermediate dark to the 2-step dark, since it involves the mutation of the one unchanged chromosome would be expected to occur at about the same rate as the change from standard to *dark 1* in the controls. Actually, the controls gave 0.7% of *dark 1* variants whereas the *gigas* intermediate darks gave 1.4% of the 2-step type. However, there is another phenomenon to be considered here. The *dark 1* type given by the controls has never been observed to revert to the standard type. On the other hand, the *gigas* intermediate dark type frequently gave a paler variant, indistinguishable from the *gigas* standard type in appearance and having the same mutational pattern. It is probable that this type results from somatic crossing over in the intermediate dark type. If this is so, a certain proportion of the 2-step darks should also result from crossing over rather than mutation. In a nucleus heterozygous for the *dark 1* mutation, crossing over between the standard chromosome and the mutated chromosome would give two daughter nuclei, one homozygous for the mutated chromosome, the other homozygous for the standard chromosome. Thus if the reversions to the standard type are due to somatic crossing over there should be a corresponding increase in the 2-step dark types. Since it was not always possible to distinguish between the paler variants corresponding to the standard type and other pale variants given by the intermediate *dark* type, they were recorded together in Table 3, and it is not possible to say how much of the increase over expectation in the 2-step dark class could be accounted for on the basis of somatic crossing over.

The frequencies of spasmodic mutants, that is those not included in the mutable series in the standard and *gigas* types are of interest. There were 0.2% of these in the controls, and 1.1 and 1.3% in the *gigas* forms. The occurrence of mutants is thus much more frequent in the *gigas* forms; it might have been expected that the *gigas* type would be less mutable than the original type on the hypothesis that the original type is haploid and the *gigas* type diploid, since mutations in one chromosome would be covered by the normal allele in the other chromosome. However, *Penicillium* normally exists in the haploid condition, and if dominance of the wild type is largely the result of natural selection, as suggested by Fisher, there is no mechanism by which such dominance could be evolved in *Penicillium*, comparable to that of diploids or of heterokaryotic haploids. In this connexion, it may be mentioned that the supposed heterozygote for the standard and *dark 1* type is intermediate, neither type being dominant. The residual mutants in the *gigas* form are mostly of reduced vigour as compared with the standard type and it is probable that they represent mutants which are lethal in the haploid and would be lethal when homozygous in the diploid.

The action of camphor on the nuclei of plants where chromosome counts

can be made without ambiguity may be considered in relation to the nature of the *gigas* forms induced by camphor treatment in yeast and other fungi. Camphor has been shown to have an effect on mitosis in onion root tips similar to the effect of colchicine (Ostergren & Levan, 1943). This has been called the *c*-mitotic effect (Levan, 1938). Levan (1947) and Levan & Sandwall (1943) have made an extensive study of the effect of camphor and other substances on the growth of yeast cells and have observed the formation of polyploid nuclei in treated material. Colchicine, the agent which is generally most efficient in inducing polyploidy in higher organisms, is ineffective on the fungi and in particular on *Penicillium* (Sansome & Bannan, 1946). The action of colchicine in inducing polyploidy is primarily due to its effect on the spindle. Since fungi have intranuclear spindles as contrasted with the extranuclear spindles of higher plants, it might be thought that the ineffectiveness of colchicine is due to the difference in spindle formation. Then one would expect that other polyploidy-inducing substances might be similarly ineffective when applied to fungi. However, *c*-mitotic substances have other effects besides their effect on the spindles as discussed in detail by Ostergren (1944). These effects include the *c*-tumour effect and an effect on chromosome contraction. When *Penicillium* and other fungi are treated with colchicine none of these effects can be recognized; the fungal growth is apparently normal. Therefore it would seem that the ineffectiveness of colchicine in inducing polyploidy in fungi is due to a general resistance to the effects of the drug rather than to the method of spindle formation. When *Penicillium* is subjected to camphor treatment, the growth is greatly affected, the hyphae are distorted, short, much branched and thickened. This effect on growth may be analogous with the *c*-tumour effect of colchicine and other substances, including camphor, on higher plants. Camphor has also a pronounced effect upon the growth of yeast as studied particularly by Levan. Cell formation is interfered with so that multinucleate hyphal-like cells are formed, and there is a tendency for the cells to stick together and form colonies.

Skovsted (1947) treated haploid cells of *Saccharomyces cerevisiae* with solutions of synthetic camphor and failed to get diploid forms, although he obtained more mutants from treated than untreated material. Bauch obtained his *gigas* forms by using natural not synthetic camphor, and Skovsted's failure to obtain diploids may be due to the fact that he used synthetic camphor. If the dextrorotatory form of camphor is more efficient than the laevorotatory form and if both forms are equally lethal, when synthetic camphor which is composed of a mixture of these two forms is used, it may be that the threshold for induction of *gigas* forms is so near that for lethality that the probability of obtaining *gigas* forms is greatly reduced. Both forms of camphor might be equally effective in producing the characteristic camphor growth forms.

Thaysen and Morris (1943) obtained *gigas* forms after camphor treatment in *Torulopsis utilis*, a non-spore-forming yeast which is probably haploid. In this fungus *gigas* forms only resulted from the treatment and there was no necessity to isolate the *gigas* from the original form. It would

seem that the *gigas* forms are more resistant to the effect of the camphor since they are able to survive doses which are lethal to the original type. In this experiment the possibility that the camphor treatment selects spontaneously occurring large mutant types has not been eliminated.

It has been suggested that some of the *gigas* forms of yeast resulting from camphor treatment may owe their increased size to a cytoplasmic effect (Levan & Ostergren, 1943; Thomas, P. T., unpublished). Levan & Ostergren suggest that dauermodifications produced by *c*-mitotic substances may be the result of a change in some lipoid-containing cytoplasmic units such as mitochondria. The hypothesis put forward by Thomas depends upon the fact that camphor treatment results in the formation of multinucleate cells. These bud off uninucleate daughter cells. It is suggested that these daughter cells of multinucleate cells are of the *gigas* type, the larger cell size being due to an effect of the cytoplasm, resulting from its having been derived from a multinucleate cell. Such an effect is supposed to persist for a time, gradually decreasing and ultimately disappearing as in the case of dauermodification.

It is difficult to see how such an explanation could hold in the case of *Penicillium* where the conidia are normally produced from multinucleate cells.

The evidence in favour of the *gigas* forms of *Penicillium* being diploid may be summarized as follows: (1) The treatment by which they have been produced has been shown to result in chromosome doubling in yeast and higher plants. (2) The difference in conidial diameter is of the order to be expected if the original type is haploid and the *gigas* type diploid. (3) The nuclei of the *gigas* type are larger than those of the original type. (4) The observations described in this paper by which the *gigas* type was found to give in two steps the change given in one step by the original type is strong genetical evidence in favour of the *gigas* type being diploid. It seems, therefore, that the *gigas* forms in *Penicillium* are almost certainly diploid and this would tend to indicate that the *gigas* forms obtained in the same way in other fungi may also be diploid. Reversion of such *gigas* forms to the original type by no means disproves the hypothesis of diploidy since occasional reduction divisions might occur and if the original form has a higher growth rate than the *gigas* form such occasional reduction divisions could result in reversion of the cultures to the original type. The difference between this type of reversion and that expected on the hypothesis of cytoplasmic effect is that the latter type would be gradual and the former abrupt. Some of the large-celled types observed in yeast which have reverted comparatively soon to the original type may well be due to cytoplasmic effects. Any such temporary effects would not have been observed in these experiments on *Penicillium* because the method of isolating *gigas* forms would tend to eliminate changes of a temporary character.

#### SUMMARY

Spontaneous mutation was studied in standard and *gigas* forms of strain 1249 B21.

The standard regularly gave a darker variant *dark* 1. The *gigas* form

similarly gave a darker variant type which was intermediate in appearance between the standard and *dark 1* types. This intermediate dark type gave variants closely resembling the *dark 1* type.

The proportions in which these types were recovered are consistent with the hypothesis that the *gigas* form is diploid and that its intermediate dark variants are heterozygous for the *dark 1* type.

The nature of the *gigas* forms is discussed in relationship to these results and to the observations of other works on yeast. It is concluded that they are probably diploids.

Thanks are due to Imperial Chemical Industries, Ltd. for financial support, and to Prof. C. W. Wardlaw for constant help and encouragement.

The text photographs were taken by Mr E. Ashby.

A Kodachrome transparency showing the differences between the standard, the intermediate dark and the *dark 1* types, and also the similarity between the *dark 1* type and the *gigas* 2-step dark has been obtained. It was hoped to produce a colour plate from this but the chance of losing these differences on reproduction is considered too great to warrant the expense of a colour plate. The original Kodachrome taken by Mr E. Young, will be deposited in the department of Cryptogamic Botany of Manchester University.

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## THE DEVELOPMENT OF FRUCTIFICATIONS OF *LENTINUS TUBER-REGIUM* FRIES, IN CULTURE

By H. BARBARA GALLEYMORE, M.Sc.

*Department of Cryptogamic Botany, University of Manchester*

(With Plate XXIV and 7 Text-figures)

In the Belgian Congo, Prof. C. W. Wardlaw found specimens of *Lentinus tuber-regium* Fries, growing among the leguminous cover crops which form part of the ground vegetation of Oil Palm plantations. This fungus produces very large spherical sclerotia which, as described by Engler and Prantl (1900), and as observed by Prof. Wardlaw, may be as large as 10 in. in diameter. Fructifications normally develop from these sclerotia (Pl. XXIV, fig. 1). One of the sclerotia was cut open, and fungal material taken from the medium-soft pseudo-tissue and transferred to 3% malt agar slopes. These cultures were brought back to Manchester and used in the work to be described.

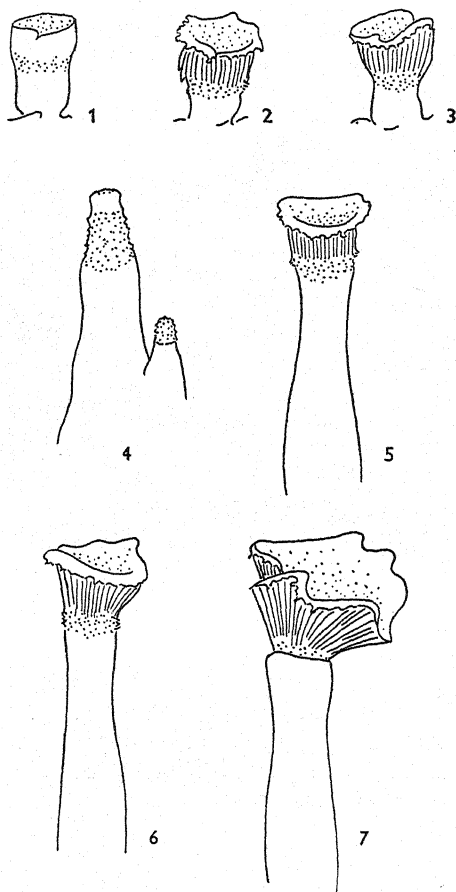
Transfers were made to various agar culture media and to different types of soils and decaying organic materials in flasks. These cultures were incubated at 24° C. in the dark. A white, fluffy mycelium grew vigorously over the surface of the agar media and spread throughout the soils, but fructifications developed only on potato agar and on riddled loam (with or without the addition of chopped leaves and stems of leguminous plants).

After three weeks' growth on potato agar, small, dark, rounded papillae, 5–10 mm. in diameter, formed in the centre of the cultures and after a further five days in the light at laboratory temperature, one of them developed into a cylindrical structure with a concave, scaly upper surface and a spotted region round the middle (Text-fig. 1). The following day, gills developed in the pale region at the top of the vertical sides of the fructification (Pl. XXIV, fig. 2; Text-figs. 2 and 3).

On the loam soils, after five to six weeks, several small, white, solid papillae formed round the sides of the flasks and after a further three weeks developed into long cylindrical rods, about 5 mm. in diameter, which grew upwards from between the soil and the sides of the flasks (Pl. XXIV, fig. 3). No further development took place in the dark. When the cultures were brought into the light, one rod in each flask became flattened on top and speckled (Text-fig. 4; Pl. XXIV, fig. 4), and after four days, a pileus with gills developed terminally on the rod which thus became the stipe (Pl. XXIV, fig. 5; Text-figs. 5–7). The pilei were funnel-shaped, pale-coloured and had dark brown scales. The edges were wavy and broken and the lamellae were decurrent, pale-coloured at first, becoming light brown. The upper parts of the long, slender stipes were also scaly.

Thus, fructifications of *Lentinus tuber-regium* were produced from mycelial cultures without the development of the large sclerotia on which the fructifications were growing under natural conditions.

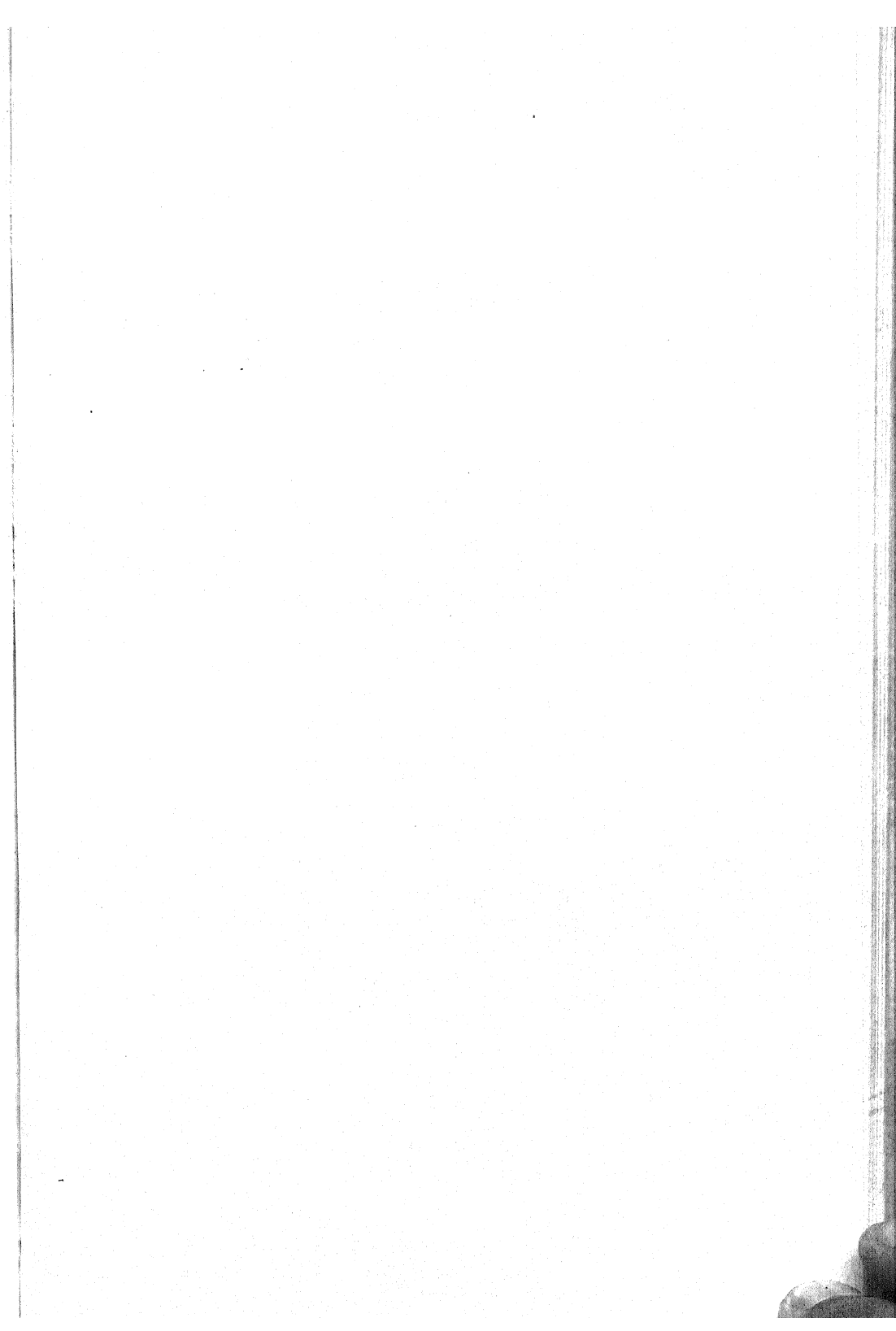
Light apparently has a formative effect on the development of the pileus of *Lentinus tuber-regium*, since in the dark the cylindrical rods continue to grow in length but do not produce pilei. Buller (1905) described the same phenomenon for *Lentinus lepideus* and suggested that sensitivity to light might be a feature of agarics which do not grow on the ground. He stated that *Agaricus campestris*, a ground agaric, usually has a substratum with



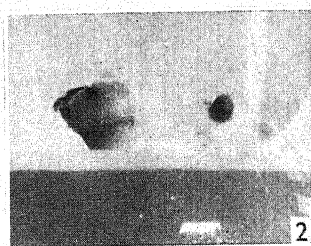
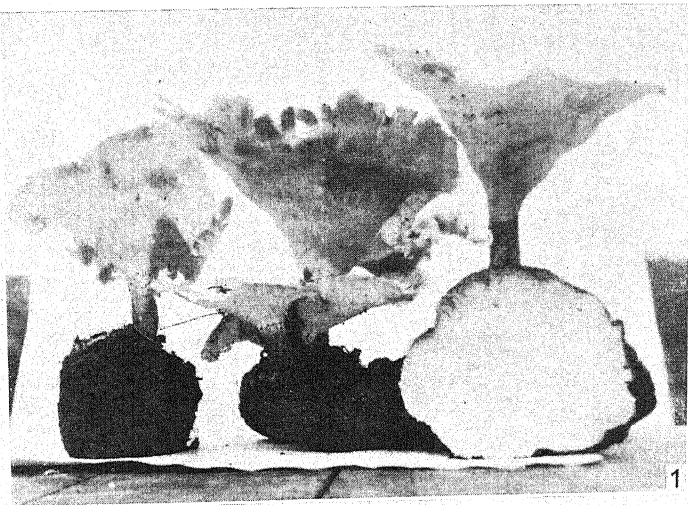
Text-figs. 1-3. Drawings showing stages in fructification formation on potato agar.

Text-figs. 4-7. Drawings showing stages in fructification formation on loam soil.

a horizontal surface, and once a fruit body has begun to form on the surface of the ground, the stimulus of gravity alone is sufficient to bring about the development of the stipe and pileus in vertical sequence with the subsequent uplifting of the latter into the air. The orientation of the surface of the substratum of *Lentinus lepideus*, a tree agaric, is, however, indefinite but response to light causes young fruit bodies to grow outwards through cracks in the bark, with the subsequent development of the pileus in the stronger light of an open space.







The reaction of *Lentinus tuber-regium* to light is not in agreement with Buller's hypothesis that all ground agarics would behave like *Agaricus campestris* with respect to the effect of light, since it, a ground agaric, is dependent on light for the formation of a pileus. A modification of Buller's hypothesis is, therefore, necessary in view of the results of this experiment.

#### SUMMARY

1. Fructifications of *Lentinus tuber-regium* normally develop on the large sclerotia which are to be found among the ground vegetation of Oil Palm plantations in the Belgian Congo.
2. A description is given of the development of fructifications from mycelial cultures without the formation of sclerotia.
3. The formative effect of light is discussed.

In conclusion I wish to thank Prof. C. W. Wardlaw and Dr A. R. Gemmell for their help and criticism, and Mr E. Ashby for his preparation of the photographs.

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#### EXPLANATION OF PLATE XXIV

- Fig. 1. Sclerotia and fructifications of *Lentinus tuber-regium* as found on Oil Palm plantations in the Belgian Congo. ( $\times$  about  $\frac{1}{2}$ .)
- Fig. 2. Fructification grown on potato agar. (Nat. size.)
- Figs. 3-5. Stages in fructification formation on loam soil. (Fig. 3  $\times$  about  $\frac{1}{2}$ . Figs. 4 and 5 nat. size.)

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# LIST OF FUNGI RECORDED AS PATHOGENIC FOR MAN AND HIGHER ANIMALS IN BRITAIN

By G. C. AINSWORTH\*

*Wellcome Trustees' Research Fellow in Medical Mycology*

## INTRODUCTION

The census of fungi recorded for Britain taken under the Society's auspices did not include 'medical fungi' because the records for these forms were widely scattered in books and journals not easily accessible to the members who undertook the preliminary search of the literature. The present list, which is an attempt to fill this gap, differs from others in the series in several ways. It is, being primarily a catalogue of pathogenic species, not confined to one taxonomic group and it includes certain actinomycetes which tend to be referred to the mycologist rather than the bacteriologist. The search of the literature (to the end of 1947) has been less thorough. Also, as medical mycologists have nothing to correspond with the *List of Common British Plant Diseases*, disease names have, whenever possible, been equated with those of important pathogens.

Study of the literature raised many problems of selection. Uncertainty as to the identity of the fungi involved necessitated the exclusion of much material. More than seventy-five papers on the dermatophytes and dermatomycoses were excluded for this reason. Uncertainty as to the pathogenicity of some of the fungi reported to have been associated with disease in man and animals led to the rejection of other records. Obvious saprophytes have been omitted and certain species included have been listed as 'isolated from' some particular organ instead of 'on' man or other animal when their status as pathogens appeared doubtful. A few poisonous fungi have also been noted. Another difficulty was to find records for even some of the common pathogens which were satisfactory from a mycological point of view. The published account of a case studied in this country has been treated as a British record even if the infection was contracted abroad. An effort has, however, been made to distinguish exotic diseases (such as mycetoma and rhinosporidiosis) from endemic diseases. Studies made in this country on non-British material have been included only when there has been a British case of the particular disease.

Most of the many text-books of bacteriology and dermatology mention fungi causing mycoses, and reference has been made to a few representative examples. These are mostly works by medical men known to have had an interest in pathogenic fungi, and reference is usually made to the latest editions which may be taken as representing the authors' considered opinions. Important clinical papers have also been compiled because clinical data cannot be ignored by the mycologist. Published notes on cases exhibited to the various medical societies have, however, been

\* *University College, Exeter.*

excluded unless they are first records for the country or provide other points of particular interest.

Attention is drawn to the fact that this list, like its companions, is not based on any extensive examination of specimens and the synonymy adopted will no doubt need to be modified. For example, the organisms causing actinomycosis in man and cattle are possibly distinct. Here they have been united under *Actinomyces israeli*. On the other hand, certain species of *Candida* which should probably be included in *C. albicans* have been treated as separate entities because they have been so distinguished in the British literature. The synonymies given for some species only include names which have been commonly used in this country. The index should be consulted for the formal author citations of the names of species mentioned incidentally in the body of the list.

#### ACKNOWLEDGEMENTS

Much of the work of compiling this list was done in the libraries of the London School of Hygiene and Tropical Medicine, the Royal Society of Medicine, and the Wellcome Historical Medical Museum. I gratefully acknowledge all that those in charge of these libraries did to lighten the task. I am also indebted to Dr J. T. Duncan and Miss E. M. Wakefield for help and advice, and to Dr P. H. Gregory for the gift of a card index to literature of the dermatophytes.

### FUNGI (EUMYCETES)

#### PHYCOMYCETES

**Absidia corymbifera** (Cohn) Sacc. & Trotter. Isolated from human sputum and nasal discharge of horses, **204** (N.S. xi, 387); from milk, **70** (N.S. xix, 314\*); **146**, 23.

**Coccidioides immitis** Rixf. & Gilchr. Coccidioidomycosis. On man (accidental pulmonary infection in a laboratory worker), Nabarro, **140** (1948 i, 982-4\*).

**Ichthyosporidium hoferi** (Plehn & Muslow) Pettit. On mackerel (esp. kidney and spleen), Sproston, **169** (xxvi, 72-98\*, 1944).

**Rhinosporidium seeberi** (Wernicke) Seeber. Rhinosporidiosis. On man (nasal polyp in an Indian medical student at Edinburgh, 1917-21), Ashworth, **53** (LIII, 301-42\*, 1923); **171** (N.S. xxx, 337-51\*) (reprinted **172** (xxxviii, 285-99\*)); **147**, 897\*. *R. seeberi* was considered by Ashworth to have chytrid affinities but this is doubtful.

**Saprolegnia ferax** (Gruith) Thuret, and **S. monoica** Pringsh. Salmon disease. On salmon and other fish: W. G. Smith, **31** (ix, 560-2\*, 1878); Huxley, **67** (xxxiii, 381-9, 1882); **68A** (N.S. xxii, 311-33, 1882, *S. monoica*); **168** (ix, 726-32); **27** (xxiii, 302-8), experimental inoculation of dace and salmon; Patterson, **199\***, *Bacillus salmonis pestis* in relation to; **219**, 1152-60. The parasite on fish and fish eggs is considered to be distinct from *S. ferax* and *S. monoica* by Coker who named it *S. parasitica* (see Kanouse, **100** (xxiv, 431-52, 1932)).

## ASCOMYCETES

- Chaetomium elatum** Kunze & Schmidt. Odour of, experimentally inducing nausea and giddiness in man, Blackley, **213**, 58.  
**Claviceps purpurea** (Fr.) Tul. Ergotism. Poisoning man, **142**, 885, 887; outbreak among Jews in Manchester, **150** (1928 I, 302), **75** (XXIX, 51, 1929); **210**.  
**Gibberella zeae** (Schw.) Petch. On barley poisoning pigs, **22** (Bull. No. 79, 18, 1934), as *G. saubinetii*.

## BASIDIOMYCETES

## USTILAGINALES

- Tilletia caries** (DC.) Tul. Spores associated with 'epileptiform convulsions' in the dog, Greig, **191** (LXXVIII, 387, 1922), **28** (x, 121), as *T. tritici*, but in feeding experiments, spores non-toxic to dogs, rabbits, and guinea-pigs, **28** (xi, 82).

## AGARICALES

- Amanita phalloides** (Fr.) Secr. Poisoning man. Fatalities, **140** (1879 II, 941; 1886 II, 1018); **150** (1905 II, 541; 1946 I, 45); **28** (III, 25, 1908). Treatment, **150** (1947 II, 348, 395). **212**, 23; **209**, 27\*; **208**, 16\*.  
**Inocybe fastigiata** (Schaeff. ex Fr.) Quél. Poisoning man, Wilson, **150** (1947 II, 297).  
**I. incarnata** Bres. Poisoning man, Young, **211** (XLIV, 52, 1925).  
**I. patouillardii** Bres. Poisoning man. Fatality in Surrey, 1937 (**208**, 28\*); **209**, 33.

## FUNGI IMPERFECTI

## MONILIALES

## PSEUDOSACCHAROMYCETACEAE

- Candida albicans** (Robin) Berkh. Moniliasis. (Syn. *Oidium albicans*, *Monilia albicans*, *Oospora albicans*, *Monilia pinoyi*; see also *Candida metalondinensis* and *C. tumefaciens* below.) On man: buccal cavity (thrush): **181**, 63\*; **161** (XXI, 221-3, 1909); **176** (CLXVII, 417, 1923); **140** (1925 II, 1226-8); **150** (1930 II, 119); **161** (XLIII, 354, 1931); **147**, 874\*; Ludlam & Henderson, **140** (1942 I, 64-70), neonatal thrush; **161** (LVII, 18-19, 1945); **150** (1945 II, 716); sputum and associated with pulmonary disorders: **140** (1923 II, 109, 110); **140** (1925 II, 1226-8); **204** (N.S. IX, 365, 1936); Shrewsbury, **203** (XXIX (N.S., v), 375-97, 1936), secondary thrush of the bronchi, *C. albicans* not considered to be a cause of primary pulmonary disease; **150** (1945 II, 715); **171** (LIII, 585; LIV, 421, 1946-7); skin: **140** (1925 II, 1226-8); **161** (XXXIX, 410, 1927); **150** (1929 II, 948); **161** (XLII, 551-9\*, 1930); **157**, 1309-17\*; **150** (1945 II, 716); nail fold (paronychia): **140** (1924 I, 128); **161** (XXXIX, 394-8, 1927; XLII, 555\*, 1930; XLIII, 140-1, 1931); nails: **161** (XXI, 221-3, 1909; XXXIX, 119-22, 1927; XLII, 557\*, 1930); **150** (1930 II, 1120); **157**, 1315\*; **161** (XLIII, 355, 1931); ear: **150** (1945 II, 716); vagina: **162** (XXXII, 358, 1929); **206**, 139; **171** (XLVII, 369\*, 1940);

- pruritus vulvae: **162** (xxviii, 245, 331, 1925); **189** (cxvii, 354, 1926), **161** (xlii, 559, 1930); pruritus ani: **162** (xxvii, 304-5, 1924; xxviii, 329-31, 1925; xli, 378, 1938). Cultural and taxonomic studies, **161** (xlii, 550, 1930); **161** (xliv, 435, 1932), Shrewsbury, **153** (xxxviii, 315, 1934); **161** (xliii, 562-8, 1930), comparison with *Monilia pinoyi*; **162** (xl, 298, 300, 1937); **153** (xxxiv, 429-36, 1931), serology; **140** (1946 i, 44-6), effect of antibiotics on; **161** (xlii, 562-8, 1930), pathogenicity to man and animals; **146**, 30; **158**, 362, 366\*, 384\*; **207**, 91. On birds: **174**, 140 (thrush in chickens); **175**, 457 (in poultry and pigeons).
- C. guillermondi** (Cast.) Langeron & Guerra. (Syn. *Endomyces guillermondi* Cast., **150** (1912 ii, 1210), *Monilia guillermondi* (Cast.) Cast. & Chalm., **205** (ed. 2, 826, 1913).) On man causing bronchomoniliasis; Jockes & Simpson, **140** (1923 ii, 110). Cultural characters, **162** (xxiii, 21, 1920; xl, 301, 1937); **207**, 246.
- C. macroglossiae** (Cast.) Castellani & Jocono, **162** (xxxvi, 314, 1933). Isolated from case of macroglossia in man by Castellani, **162** (xxviii, 218 (as *Cryptococcus macroglossiae*), 220\* (as *Monilia macroglossiae*), 1925); **206**, 21; **144** (xxi, Sect. Trop. Dis. Parasitol. 3, 1928); Castellani & Jocono, **162** (xxxvi, 314\*, 1933), as *Torulopsis macroglossiae*; Castellani, **162** (xl, 306, 1937), as *Monilia zeylanoides* var. *macroglossiae*. [= *C. zeylanoides* vide **207**, 298.]
- C. metalondinensis** (Cast.) Berkhout. (Syn. *Monilia metalondinensis* Cast. in **205** (ed. 3, 1082, 1919, as 'Castellani 1916'.)) On man: tonsils, Castellani, Douglas & Thompson, **162** (xxvi, 23, 1923), **206**, 113, 115, **189** (cxxxiv, 74\*, 1930), **144** (xxi, Sect. Trop. Dis. Parasitol. 449, 1928; and nose); vagina, **162** (xxxii, 358, 1929), **206**, 139; sputum, **206**, 123, **162** (xxxii, 5, 1929); **153** (xxxiv, 433, 1931), serology; **162** (xxiii, 21, 1920; xl, 300, 1937), cultural characters; **206**, 123, pathogenicity for rabbit. [= *C. albicans* vide **207**, 93.]
- C. tropicalis** (Cast.) Basgal. (Syn. *Oidium tropicale* Castellani, **150** (1910 ii, 869); *Endomyces tropicalis* (Cast.) Cast.; *Monilia tropicalis* (Cast.) Castellani & Chalmers, **205** (ed. 2, 824, 1913, as 'Castellani 1909'.)) On man causing thrush, Craik, **176** (clxvii, 417, 1923, as *M. candida*); vaginitis, **206**, 139, **162** (xxxii, 358, 1929); dermal moniliasis, **150** (1945 ii, 716); in sputum, **162** (xxxviii, 244-5, 1935), **150** (1945 ii, 715). Cultural characters, **162** (xxiii, 21, 1920; xxiv, 150\*, 1921; xxviii, 258\*, 1925), **153** (xxxviii, 315\*, 1934), **162** (xl, 299\*, 1937); serology, **153** (xxxiv, 433, 1931).
- C. tumefaciens** (Foulerton) C. W. Dodge. Isolated from two cases of pharyngitis (associated with *Corynebacterium diphtheriae*), Foulerton, **153** (vi, 49, 1900), as *Saccharomyces tumefaciens albus*; **143** (i, 351, 1899), pathogenic for the rabbit. [= *C. albicans* vide **207**, 91.]
- C. zeylanoides** (Cast.) Langeron & Guerra. (Syn. *Monilia zeylanoides* Castellani, **162** (xxiii, 17, 1920, as 'Cast. 1917' on p. 21); **162** (xxviii, 257-8, 1925).) On man (moniliasis of toes), Castellani, **162** (xlii, 293-5\*, 1939; xliii, 81, 1940). Cultural characters, **162** (xl, 306, 1937), **207**, 298. See also *C. macroglossiae*.

**Cryptococcus farciminosus** Rivolta. [*Histoplasma farciminosum*] Epizootic lymphangitis. On the horse. Introduced into this country after the South African War, last cases destroyed 1906, Pallin, **167**; **217**, 54; **219**, 1289-1301; **151**, 407-9\*. Scheduled in Great Britain under the Diseases of Animals Acts.

**C. gracilioides** Castellani, **162** (xxviii, 222, 1925). On man causing stomatitis (associated with *Bacillus vermiculoides* Cast.); **162** (xxix, 224, 1926); **206**, 21, 110. [= *Pityrosporum ovale* vide Ota & Huang, *Ann. parasitol. hum. comp.* xi, 49, 1933.]

**Malassezia furfur** (Robin) Baillon. *Tinea versicolor*. (Syn. *Microsporon furfur*.) On man: **181**, 57\*; **182**, 158; **214**, 463; **156**, 70-1\*; **150** (1929 II, 950); **161** (xlii, 559-61, 1930); **157**, 440-3\*; **147**, 884\*; **158**, 363, 388; **159**, 405-7\*.

**Monilia batrachea** Scott, **154** (1926, 692), on bull frogs (*Rana catesbiana*), black spotted toad (*Bufo melanostictus*), and other amphibians; **154** (1928, 119), on tigrine frog (kidney, liver, and lungs).

**M. londinensis** Cast. in **205** (ed. 3, 1082, 1084, 1919, as 'Castellani 1916'), isolated from case of thrush in man; **162** (xxiii, 21).

**Pityrosporum ovale** (Bizz.) Cast. & Chalm. in **205** (ed. 2, 832, 1913, as 'Bizzozzero 1882'). On man (associated with dandruff), **156**, 72-6\*, **150** (1929 II, 950), in relation to seborrhoeic dermatitis; **161** (xlii, 559, 1930); **150** (1930 II, 1121); **161** (xlix, 74, 1937); **158**, 366\*, 386-8; **180**, 158\*. See also *Cryptococcus gracilioides*. (The organism studied by Macleod & Dowling, **161** (xl, 139-48, 1928) and **157**, 1317 was not *P. ovale*, vide Dowling **161** (li, 7, 1939).)

**Torulopsis neoformans** (Sanf.) Red. Torulosis. (Syn. *Cryptococcus neoformans*, *Torula histolytica*.) On man: Smith & Crawford, **153** (xxxiii, 291-6\*, 1930); **140** (1938 II, 1154-7\*), as *Cryptococcus meningitidis*; **140** (1942 II, 394); **150** (1945 II, 715, 716), as *Debaryomyces neoformans*; **150** (1948 I, 1082).

**Trichosporon beigeli** (Rabenh.) Vuill. White piedra. On man (hair): Pye-Smith, **143** (xxx, 439-41, 1879); Cheadle & Morris, **140** (1879 I, 190); Pernet, **161** (xii, 141, 1900); **161** (xxiv, 131-7\*, 1912), study of material from British Guiana; **161** (xxxiv, 265, 1922), reprinted, **162** (xxvi, 190, 1923); **206**, 147; **157**, 437-9\*.

#### MONILIACEAE

**Alternaria tenuis** Nees. Spores as air-borne allergens. Daily incidence of *Alternaria* spores at Cardiff, 1942-3, Hyde & Williams, **28** (xxix, 78-85, 1946).

**Aspergillus flavus** Link. On man (otomycosis), Duncan, **150** (1945 II, 717).

**A. fumigatus** Fresen. Aspergillosis. On man: lung, Delikat, **140** (1945 II, 370-1\*) and probably **143** (v, 38-41, 1854) and **143** (xli, 34-7, 1890); sputum, **143** (L, 349-50, 353), **150** (1945 II, 715); nasal sinuses, **144** (xii, Laryngol. 187-8); vagina, **162** (xxviii, 246). **140** (1942, I, 394); enzymes, **173** (xxi, 36); serology, **28** (xiv, 73). On birds in captivity: flamingo (lung), Owen, **154** (II, 142, 1832), as 'green



vegetable mould or *mucor*'; **143** (L, 357); **154** (1910, 134), 48 birds, 1 reptile at the London Zoo; **154** (1926, 178-9), contaminated grain in relation to; **145** (CXLIX, 247, 251, 255), association with tuberculosis; **150** (1945 II, 717). Poultry (black-mould pneumonia, brooder pneumonia), **174**, 139-40; **151**, 414-16; **175**, 454-6. On other animals; monkey (spleen), **154** (1928, 90); bison (lung), **61** (CLX, 362); horse (lung), **143** (L, 272). Pathogenic for rabbit, **143** (xxxvii, 549), Rothwell, **153** (vii, 34-52, 1901), and review of earlier literature.

**A. niger** van Tiegh. On man: Cooke, **14** (vi, 127), **73** (Ser. 2, II, 140\*, 1885), as *A. nigricans*, causing otomycosis; **8** (III, 298); Delépine, **143** (XLII, 423-57\*, 1891), associated with melano-mycosis of the skin. On birds: **154** (1912, 239); **145** (CXLIX, 251, 1930); **164** (XLIX, 680); **154** (1926, 178-9), contaminated grain in relation to. Rothwell, **153** (vii, 34, 1901), pathogenic but never fatal for rabbit and guinea-pig; **173** (xxi, 31-54, 1929), enzymes; **28** (xiv, 73), serology.

**Blastomyces dermatitidis** Gilchrist & Stokes. North American Blastomycosis. On man: Dowling & Ellsworthy, **144** (xix, Dermat. Sect. 4-10\*, 1925), nail fold then systemic; Castellani, **144** (xxi, Sect. Trop. Dis. Parasitol. 449, 1928), mouth (infection contracted in Egypt), as *B. tulanensis*; **161** (LV, 294-6, 1943), leg; **150** (1945 II, 716), arm (infection contracted abroad); **150** (1930 II, 1122); **140** (1942 I, 292).

**Corethropsis hominis** Vuill. On man (tinea pedis), Fraser, **162** (XLII, 144, 1939).

**Fusarium oxysporum** Schlecht. ex Fr. Forming a fungal cast of stomach in man (from Africa), Duncan & Murgatroyd, **155** (xxxii, 6-7, 1938), as *Fusarium* sp., since identified as *F. oxysporum*.

**Geotrichum pulmoneum** (Catt. & Oliva) Basgal. Isolated from human sputum and lung by Bennett, **53** (xv, 282\*, 1842), but fungus not named; **216**, 515\*; **182**, 224\*; as '*Oidium pulmoneum* Bennett', Cattaneo & Oliva, *Archivo Lab. Bot. Crittogram. Univ. Pavia*, v, 126, 1888 [preface dated 1883]; **8** (III, 278); as *Oospora pulmonea* '(Bennett) Saccardo', *Sylloge*, iv, 16, 1886.

**G. rotundatum** (Cast.) Red. (Syn. *Endomyces rotundus* Castellani, **150** (1912 II, 1209); *Monilia rotunda* Cast. & Chalm., **205** (ed. 2, 828); *M. rotundata* (Cast.) Cast., **162** (xvii, 307, 1914); *Oidium rotundatum* (Cast.) Cast. & Chalm., **205** (ed. 3, 1095).) On man: tonsils, Castellani, **206** (II, 15, 1928); toes, **162** (80\*, 1940), infection probably contracted in India.

**G. rotundatum** (Cast.) Red. var. **gallicum** Castellani, **162** (81\*, 1940), on man (toes), infection probably contracted in France.

**G. rugosum** (Cast.) C. W. Dodge, **152**, 219. (Syn. *Endomyces rugosa* Castellani, **150** (1912 II, 1210); *Monilia rugosa* [Cast.] Cast. & Chalm., **205** (ed. 2, 827), *Hemispora rugosa* (Cast.) Cast. & Chalm., **205** (ed. 3, 1108, as 'Castellani, 1910').) On man: sputum, Castellani, Douglas & Thompson, **162** (xxiv, 149\*, 1921), causing 'bronchohemisporosis'; **206**, 128; tonsils, **162** (xxvi, 24, 1923), **206**, 117, **162** (xxviii, 247-8\*, 1925), and vagina; **162** (xxxii, 19\*, 1929).



- Glenospora semoni** Chalmers & Archibald. On man (mycetoma pedis in an Indian soldier), Semon, **161** (xxvii, 299-303, 1915).
- Hemispora pararugosa** Castellani, Douglas & Thompson, **162** (xxiv, 149\*, 1921), from human sputum, causing 'bronchohemisporosis'; **206**, 128; Castellani, **162** (xxxii, 19\*, 1929), as *H. rugosa* var. *pararugosa*. **152**, 225, as *Mycoderma pararugosa* (Cast., Dougl. [& Thomps.]) C. W. Dodge.
- Histoplasma capsulatum** Darling. Histoplasmosis. On man: Derry & Card, **140** (1942 I, 224-7\*); Duncan, **140** (1942 I, 394); a variant form, Duncan, **150** (1945 II, 716), **155** (XL, 364).
- Penicillium 'glaucum Link'**. Spores experimentally inhaled inducing bronchial catarrh in man, Blackley, **213**, 58.
- Sporotrichum anglicum** Castellani, **162** (XL, 315\*, 1937), from human sputum.
- S. schencki** (Hekt. & Perk.) Matr. Sporotrichosis. (Syn. *S. beurmani*.) On man: Walker & Ritchie, **150** (1911 II, 1-5\*), first British case; **144** (IV, Dermat. Sect. 113-21\*, 1911), infection contracted in Brazil; **161** (xxv, 33-7, 60-4, 1913); **161** (xxix, 270-3\*, 1917), boy in Dublin; **161** (xxx, 174, 1918); **140** (1942 I, 393), **150** (1945 II, 716), knee joint; **143** (LII, 259-70\*, 1901), cultural study; **161** (xx, 296-303, 1908), and **150** (1912 II, 289-96), reviews; **157**, 433-7\* (skin); **146**, 43; **147**, 877\*; **158**, 389\*.

## DERMATOPHYTES

Many clinical and doubtful records have been excluded. Reference is made to representative books on dermatology (**156**, **157**, **158**, **159**, **193**, **214**), and on diseases of the hair (**180**, **195**), but these works have not been indexed exhaustively. The complementary reviews of this group by Tate, **188** (IV, 41, 1929) and Gregory, **188** (x, 208, 1935), and the general account by Ramsbottom & Whitfield, **146**, 46, 1931 may also be consulted.

**Achorion gallinae** (Mégnin) Sab. Fowl Favus ('white comb'). (Syn. *Lophophyton gallinae*.) On fowls: **22** (Leaflet No. 67, 1908); **150** (1928 I, 656) and **157**, 381, as *Trichophyton rosaceum*; **174**, 138-9; **151**, 414; **175**, 456. This species appears never to have been transferred to *Trichophyton* where it presumably belongs.

**Epidermophyton floccosum** (Harz) Langeron & Milochiev. Epidermophytosis. (Syn. *E. cruris*, *E. inguinale*.) On man: Whitfield, **144** (IV, Dermat. Sect. 111-12, 1911); **161** (xxiii, 375-402, 1911 and **144**, v, Dermat. Sect. 36-62, 1912) ['Whitfield's ointment']; **156**, 54; **140** (1922 II, 791), control; **150** (1923 II, 1041); **162** (xxviii, 245, 329, 1925), **189** (cxvii, 343, 345, 354, 1926), **206**, 167, 171, **162** (xli, 378, 1938), pruritis ani and vulvae; **150** (1928 I, 656); Castellani, **140** (1928 II, 595) ['Castellani's paint']; **176** (clxxvi, 219, 1928); **157**, 393\*, 395, **418** (nails); **147**, 870; viability in detached scales, **162** (xli, 311, 1938); incidence in the navy, **162** (xlii, 144, 1939), in boys' schools, **145** (ccxxvii, 223-5, 1938); treatment, **162** (xxxvii, 353, 1934), **158**, 361, 362, 384\*, 393 (and **179**, III, 166); **150** (1940 I, 739); cultivation, **177** (IV, 94); **177** (V, 78); **150** (1945 II, 717, 718); **177** (V, 100); **178**, Fig. 139 a.

**Microsporum audouini** Gruby. Human Microsporiasis. On man: (children), Tilbury Fox, **181**, 57\*, 1863; **214**, 461; **182**, 153\*; Adamson, **161** (vii, 206-11, 1895), 'Adamson's fringe' (pls. 1, 3 and 4); Colcott Fox & Blaxall, **161** (viii, 243\*, 1896), **143** (xlviii, 301-3\*, 1897); **183**, 19, 73-82, pl. 1; **184**, 140\*; **186**, 551; **195**, 50; **193**, 70; **150** (1928 I, 656); **157**, 376-7\*, 398-400\* (clinical); **147**, 865\*; **158**, 363 (trunk), 364 (scalp), 375\*, 392 (and **179** (iii, 166, 1945)); **180**, 179; **177** (iii, 168); **150** (1945 II, 717); **192** (1944-5, 96\*); (adults) **161** (xxi, 90-1 (and **144** (ii (1), Derm. Sect. 89-90)); xxiii, 84-5; xxix, 114; xlv, 242). Incidence in London, **143** (xlviii, 301, 1897); **186**, 529, 573; in London County Council Schools, **75** (xxvii, 32-6, 1927); in Edinburgh, **186**, 529; **150** (1932 I, 93-4), of 'tinea capitis', 1912-30; in England, **150** (1945 II, 346-9); epidemic in Scotland, **187** (v, 66-8, 1947); detection by ultra-violet light, **150** (1931 I, 791-2), **177** (iii, 170); X-ray epilation, **161** (xv, 66-8, 1903; xxii, 46-9, 1910; xl, 440-50, 1938); thallium acetate epilation, **161** (xxxix, 307, 1927; xliii, 59-69, 1930); **150** (1932 I, 8-10); lice, as carriers of, **140** (1920 I, 981, 1932); infection of hair, Fox & Blaxall, **150** (1899 II, 1529-32\*); experiments with microsporin, **215**, 129, 146; enzymes, **173** (xxi, 31-54, 1929); staining, **189** (lv, 135-7\*, 1895); culture, **161** (xxxvi, 204-6), **177** (iv, 94). See also *Trichophyton tonsurans* sensu lato.

**M. canis** Bodin. Cat and Dog Microsporiasis. (Syn. *M. felineum*, *M. lanosum*.) On man: Fox & Blaxall, **161** (viii, 352-4\*, 381-3, 1896), first description of *M. canis* but fungus not named; incidence in London County Council Schools, **75** (xxvii, 32-6, 1927); in England, **150** (1945 II, 717; 1947 II, 896); infection from cats and dogs, **161** (x, 37\*, 1898; xiv, 327, 1902), **190** (xx, 58, 1912), **191** (lxxxviii, 439, 1922; lxxxv, 303, 306, 1927), **150** (1945 II, 346), **218** (lxxvii, 65); tinea capitis in an adult, **161** (xxvii, 119, 1920; xxix, 114, 1927); cultivation, **177** (iv, 94); enzymes, **173** (xxi, 31, 1929). **150** (1928 I, 656); **157**, 378\*; **158**, 363, 364, 376\*, 392 (and **179**, iii, 166); **192** (1944-5, 96\*); **180**, 179; **178**, Figs. 136, 137 a, b, 140. On cats and dogs: **161** (x, 37\*, 1898), T.S. and V.S. of cat skin; **206**, 58; **151**, 411. See also under man above.

**M. domesticum** Webb in Macleod & Muende, **158**, 164, 376-7\*, 392, on man (tinea capitis in children) and the cat.

**M. equinum** (Delac. & Bodin) Guég. On man and the horse: Macleod, **150** (1928 I, 656); **178**, Fig. 137 c; **147**, 865; **151**, 411.

**M. gypseum** (Bodin) Brumpt. On man: Macleod & Muende, **158**, 375, as *M. fulvum* syn. *Achorion gypseum*.

**Trichophyton concentricum** Blanch. Tinea imbricata. On man (European): infection first noticed when in Arabia, Castellani, **162** (xxxvii, 363-7\*, 1934), **144** (xxvi, 121), **161** (xlv, 23-4), **176**, 17 May 1933), as *Endodermophyton indicum*; **206**, 171\*; **158**, 389.

**T. discoides** Sab. Calf ringworm. On man (and calves): Thomas *et al.* **150** (1945 II, 347), **150** (1945 II, 717, 718); **177** (iv, 94) cultivation; **140** (1932 I, 97-9).

**T. equinum** Geddoelst. On the horse: Macleod, **150** (1928 I, 656); **151**, 411.

- T. flavum** Bodin. On man: Macleod & Muende, **158**, 381\*, 392; **179** (III, 166), as *T. cerebriforme*.
- T. mentagrophytes** (Robin) Blanch. (Syn. *T. asteroides*, *T. gypseum*, *T. interdigitale*, *T. niveum*, *T. pedis*. See also *T. tonsurans* sensu latu.) On man: Neligan, **194** (pl. 16, fig. 14, as *Microsporon mentagrophytes*; pl. 15, fig. 1, sycosis); **182**, 155; **181**, 57\*, sycosis; Tilbury Fox, **140** (1873 II, 141-2\*), **214**, 458, sycosis; **8** (II, 309), as *Sporotrichum mentagrophytes*; Colcott Fox & Blaxall, **143** (XLVIII, pl. 11, fig. 2) and on cats, as 'an ectothrix fungus'; Adamson, **161** (xx, 127, 1908), as 'Trichophyton megalosporon ectothrix du cheval à cultures blanches'; **153** (XII, 232-6\*, 1908), infection contracted from calves; **176** (CLXXVI, 222, 1928), tinea pedis, as *Trichophyton niveum*; **161** (XLIII, 121, 1931), erythema multiforme; **145** (CCXXVII, 223, 1938), tinea pedis in boys' schools; **162** (XLII, 144, 1938), tinea pedis in the navy; **158**, 362, 363, 378\*, 393 (and **179** (III, 166)); **150** (1940 I, 739); **150** (1945 II, 717); **177** (v, 76), tinea pedis; **150** (1945 II, 347, 348), tinea circinata (from contact with calves and cats) and tinea tonsurans; **177** (iv, 94), cultivation; enzymes, **173** (XXI, 31-54, as *Sabouraudites radiolatus*); **28** (XXI, 98), spiral hyphae; **192** (1944-5, 96\*). On cattle: Muende & Webb, **197** (XXXVI, 987), and growing saprophytically on dung; **217**, 685. See also under man above. On cats: as *Trichophyton felinum*, Gray, **191** (LXXIX, 314, 1923), **151**, 411; as *T. niveum*, **150** (1928 I, 656). See also under man above. On the dog: Macleod, **150** (1928 I, 656), as *T. niveum*. On the horse: **217**, 684. On the mouse: Parish & Craddock, **196** (XII, 209, 1931), ringworm epizootic which spread to four laboratory attendants.
- T. ochraceum** Sab. On man: **157**, 382; **180**, 185, beard.
- T. persicolor** Sab. On man: Adamson (vide **198**, 371), as 'the peach-coloured trichophyton'; **157**, 381; **158**, 379\*, as *T. purpureum*; **150** (1945 II, 717); **177** (iv, 94), cultivation.
- T. plicatile** Sab. On man: Sequeira, **161** (XVIII, 269, 1906; XXIV, 207-15\*, 1912) and **159**, 404, trichophytic granulomata of the umbilicus; **157**, 415, tinea barbae; **159**, 392. [This species is probably *T. epilans* Mégnin.].
- T. quinckeanum** (Zopf) Macleod & Muende. Mouse favus. (Syn. *Achorion quinckeanum*.) On man (and the mouse): Roberts (**185**, 22-3), the 'achorion of Quincke' said to be seldom met with in Britain; Adamson, **144** (II, Dermat. Sect. 1-3\*, 1909 and **161** (xx, 365)), **161** (XXI, 116-17), **144** (II, Dermat. Sect. 91, 103-4\*), mouse and child; **161** (XXV, 138, 165, 1913); **161** (XXVI, 84 (child), 323 (mouse), 1914), **161** (XXXVI, 168, 1924); **150** (1929 II, 1194\*); **157**, 425\*; **147**, 869\*; **158**, 363, 383\*, 393 (and **179**, III, 166); **150** (1945 II, 717); **177** (iv, 94); **159**, 405\*.
- T. roseum** Bodin. On man: beard: Adamson, **161** (xx, 258, 1908), as 'Trichophyton ectothrix à cultures roses' (= *T. rosaceum* vide **198**, 258); **144** (II, Dermat. Sect. 83-4, 161, 1909); **161** (XXI, 325, 1909); Bolam, **161** (XXIV, 1-13\*, 1912), general account and 16 cases of tinea barbae and tinea circinata; **156**, 58; **157**, 381\*; **147**, 866\*; nails, **171** (N.S. VI,

125\*, 1911), 159, 404; feet, 150 (1945 II, 717); 177 (IV, 94, 1945), cultivation. [This species is probably *T. magnini* Blanch.]

**T. rubrum** (Cast.) Sab. (Syn. *Epidermophyton rubrum*.) On man: Semon, 161 (XXXIV, 355, 397-400\*, 1922), nails; Castellani, 150 (1923 II, 1041), 189, (CXVII, 343, 345, 1926), 162 (XLI, 378, 1938), pruritus ani; tinea pedis, 162 (XLI, 144, 1939), in the navy, 150 (1945 II, 717, 718), 177 (V, 78, 1946); 177 (IV, 94, 1945), cultivation; 147, 870\*; 158, 362 (groin), 379\*, 393 (and 179, III, 166, 1945), as *Trichophyton purpureum*.

**T. sabouraudi** Blanch. (Syn. *T. acuminatum*.) On man: Colcott Fox, 144 (II, Dermat. Sect. 58\* and 161, XXI, 283, 1909); 171 (N.S. VI, 125\*, 1911); experiments with trichophytin, 215, 130; 75 (XXVII, 32-6, 1927), incidence in London County Council Schools; 150 (1928 I, 656); 157, 380\*, 415, tinea barbae; 158, 364, 380\*, 392 (and 179 (III, 166, 1945)); 150 (1945 II, 349, 717, 718), hair and nails; 177 (IV, 94, 1945), cultivation.

**T. schoenleini** (Leb.) Langeron & Milochev. Favus. (Syn. *Achorion schoenleini*.) On man: Bennett, 200 (II, 504-19\*, 1842) and 53 (XV, 277\*, 1842), the fungus nature of favus cups described but fungus not named; 194 (pl. 16, fig. 5; pl. 15, figs. 2 and 3); 182, 162; 181, 56, pl. 1, fig. 1); 201, 50, 56; 214, 429; as *Oidium porriginis*, 19, no. 546, 15, 604, 18, 350; as *Oospora porriginis*, 8 (III, 278); as *Achorion vulgaris*, 185, 11, 19; 161 (III, 101-5\*, 1891); 184, 139, fig. 16; 195, 60; 193, 92; 171 (N.S. VI, 126, 1911), nails; 156, 60-2\*; 75 (XXVII, 32-6, 1927), incidence in London County Council Schools; 215, 131, experiments with favin; 173 (XXI, 31-54, 1929), enzymes; 150 (1931 I, 792), detection by ultra-violet light; 161 (XLIV, 500, 1932; LIV, 237, 1942); 157, 421-9\*, 147, 869\*; 158, 363 (trunk), 364 (scalp), 383\*, 393 (and 179 (III, 166, 1945)); 180, 190; 150 (1945 II, 717, 718); 192 (1944-5, 96\*); 177 (IV, 94, 1945), cultivation; 178, fig. 133, 139 a.

**T. sulphureum** Colcott Fox. On man: Colcott Fox, 144 (II, Dermat. Sect. 57\*, as 'the primrose crater'), reprinted, 161 (XXI, 280\*, 1909, as *T. sulphureum*), hair; Low, 171 (N.S. VI, 125\*, 1911), nails (as *T. crateriforme flavum*); 161 (XXVI, 431, 1914), tinea circinata; 215, 130, experiments with trichophytin (as *T. crateriforme flavum*); 75 (XXVII, 32-6, 1927), incidence in London County Council Schools; 150 (1928 I, 656); 157, 380\*; 147, 866\*, (as *T. crateriforme flavum*), causing ringworm of the scalp in Scotland; 158, 380\*, 392 (and 179, III, 166, 1945); 150 (1945 II, 717).

**T. tonsurans** Malmst. (Syn. *T. crateriforme*.) On man: Fox & Blaxall, 143 (XLVIII, pl. 13, fig. 1), Fox, 144 (II, Dermat. Sect. 55\* (and 161, XXI, 279\*), 1909); 75 (XXVII, 32-6, 1927), incidence in London County Council Schools; 173 (XXI, 31-51, 1929), enzymes; 150 (1928 I, 656); 157, 380\*, 415 (beard), 418 (nails); 158, 364, 380\*, 392 (and 179, III, 166, 1945); 150 (1945 II, 347 (tinea circinata), 717 (and tinea capitis)); 177 (IV, 94, 1945), cultivation.

During the latter half of the nineteenth century (and occasionally since) *T. tonsurans* was frequently used in a wide sense to cover all species of *Trichophyton* or even all ringworm fungi. The following references have a historical interest: 182, 140\*; 181, 56, pl. 1, fig. 2;

- 201, 20, 56; 214, 432; Thin, 202, 163 (LXI, 179-88, 1878), demonstration of fungus in skin of horse, 67 (XXXIII, 234-46, 1881), experiments in artificial cultivation; Taylor, 163 (LXII, 177-88, 1879), T.S. and L.S. of infected human hairs; Roberts, 161 (I, 359-65, 1889), artificial cultivation, 185, 10, 15\*, *T. capitis vulgaris* n.nom. for *T. tonsurans* in the wide sense; 22 (Leaflet No. 95, 1908), on cattle.
- T. tenuishypha** Castellani, 162 (XLII, 376, 1939). On man (hand, arm, trunk), London.
- T. violaceum** Bodin. On man: Colcott Fox, 144 (II, Dermat. Sect. 62\* (and 161, XXI, 285\*), 1909); 156, 58 (beard); 75 (XXVII, 32-6, 1927), incidence in London County Council Schools; 150 (1928 I, 656), beard and scalp; 157, 382\*, 415 (beard), 418 (nails); 158, 364, 381\*, 393 (and 179, III, 166, 1945).

## BACTERIA (SCHIZOMYCETES)

## ACTINOMYCETALES

- Actinomyces gibsoni** C. W. Dodge, 1935 (August) (152, 722). Isolated from enlarged human spleen, Gibson, 153 (XXXIII, 357-8, 1920), but not named. Cultural characters described and tentatively designated '*A. gibsonii*', Erikson, 145 (no. 203, 36, 9, 15, Sept. 1935). [Aerobic. *Streptomyces gibsonii*.]
- A. graminis** Topley & Wilson, 148 (ed. 1, pp. 242, 250, 1929), the aerobic *A. bovis* of Bostroem, is a saprophyte of grains and grasses sometimes found associated with man and animals. *Streptothrix bovis communis* Foulerton, 143 (LIII, 87, 95) and perhaps other records included under *Actinomyces israeli* (q.v.) probably belong here.
- A. israeli** (Kruse) Lachn.-Sand. Actinomycosis. [Actinomycosis in man and cattle is frequently attributed to *A. bovis* Harz, but because this binomial is a nomen confusum (see Erikson, 145 (no. 240, 46)), it is preferable to designate the cause of actinomycosis in man *A. israeli*. From a study of Australian material, Erikson (loc. cit.) concluded that actinomycosis in cattle is caused by a distinct but allied species. Here the records for anaerobic forms from man and animals have been united under *A. israeli*. See also *A. graminis*.] On man: Shattock, 143 (XXXVI, 254-61, 1885) appears to be the first published British record (liver, diagnosed post-mortem), but Acland, 143 (XXXVII, 546-9, 1886) claimed to have first recognized actinomycosis in this country, early in 1885. Powell *et al.* 163 (LXXII, 175-208, 1889) were the first in Britain to diagnose pulmonary actinomycosis during life. 143 (XL, 408-43), as *A. hominis*; 163 (LXXV, 63-84\*), appendix; 163 (LXXV, 85-102\*), urinary and alimentary tracts; 163 (LXXV, 103-7), face and neck; Foulerton, 140 (1899 II, 779), as *Streptothrix actinomycotica*, 149 (XIV, 50, 53\*), as *S. bovis communis* [n.nom. for *Actinomyces bovis*], 140 (1905 I, 1200), 142, 308, as *Streptothrix hominis* III [*Actinomyces bovis* vide Chalm. & Christoph. 141 (X, 256)], 140 (1910 I, 553\*, 626-7), as '*Streptothrix hominis* IV'; 153 (XIV, 164-73), central nervous system; 165 (I, 197-212); 150 (1920 I, 435), cultivation; 161 (XLVI, 12-19), skin,

*Bacillus actinomycetum comitans* in relation to (see also **150** (1921 I, 893); **146**, 85-6); **157**, 429-33\*, skin; **166**, 16-24\*, biological characters; **158**, 352, skin; Erikson, **145** (no. 240), taxonomy; **150** (1945 II, 715, 716, 717), central nervous system, lung, appendix; **140** (1948 I, 439), liver with recovery. Treatment with vaccine (actinomycotin), **150** (1908 I, 554-7), **150** (1913 I, 991-2), **150** (1916 II, 488), sulphonamides, **140** (1940 II, 707), **150** (1943 II, 106); penicillin, **150** (1945 II, 728). Historical and general surveys, **150** (1889, 1339-44); **140** (1897 I, 11-18); **142**, 324-43; **146**, 78-85; **166**; **147**, 473\*; **148** (ed. 3, 379, 382-3, 1270-4). On cattle: MacFadyean, **150** (1889, 1339-44); **140** (1897 I, 11-18); **149** (xx, 189), incidence in Britain; **150** (1922 II, 1163-4), vaccine treatment; **149** (xxxvi, 1, 1923), as *Streptothrix israeli*; **217**, 143; **151**, 311-16\*. **75** (xv, 195-207), confusion with *Actinobacillus* (see **146**, 87); **164** (N.S. XII, 55-9; xv, 15-17), 'actinomycotic' mastitis caused by *Staphylococcus pyogenes*. On the cat: Edington, **164** (N.S. XIV, 311-12, 1934). On the pig: Davies & Torrance, **149** (xliii, 220), as *Streptothrix actinomycetes*.

**A. muris-ratti** (Schottmüller) Topley & Wilson, **148** (ed. 1, 251, 1929). On the rat (nasopharynx): Strangeways, **153** (xxxvii, 45-51, 1933), as *Streptobacillus moniliformis*. *Actinomyces muris* Topley & Wilson, **148** (ed. 2, 251, 1936) n.nom. for *Streptothrix muris-ratti*; ibid. ed. 3\*, 285, 1276-8. [Aerobic.]

**Micromonospora fusca** Jensen. Isolated by Duncan from diseased hair, Erikson **145** (no. 240, 28).

**Nocardia appendicis** Chalmers & Christopherson, 1916 (**141**, x, 256), n.nom. for *Streptothrix hominis* iv, Foulerton (**140**, 1906, I, 970), from human appendix. Foulerton, **142**, 308; **140** (1910 I, 553\*, 626-7, as '*S. hominis* III').

**N. asteroides** (Eppinger) Blanchard. (Syn. *Actinomyces asteroides*, *Streptothrix eppingeri*.) On man: Foulerton & Jones, **143** (LIII, 87, 97, 1902); **142**, 308, **140** (1910 I, 551, 629\*, 769); **144** (vi, Surg. Sect. 138) and **140** (1913 I, 382); **146** (VIII, 75); **145** (no. 203, 10, 26); **147**, 477; **148** (ed. 3, 388).

**N. caprae** (Silberschmidt) Emmons. (Syn. *Actinomyces caprae* (Silberschmidt) Ford.) On man (spleen in two cases (brother and sister) of acholuric jaundice), Gibson *et al.* **150** (1938 I, 612-14); **149** (xiv, 56\*), **143** (LIII, 87, 99), **142**, 308, **140** (1910 I, 556), as *Streptothrix caprae*; **145** (no. 203, 37, 26), cultural characters; **148** (ed. 3, 387). On the horse (sub-maxillary abscess), Dean, **143** (LI, 26-47, 1900); organism not named but *Actinomyces caprae* group vide **148** (ed. 3, 1276).

**N. foulertoni** Chalmers & Christopherson, (**141**, x, 256, 1916), n.nom. for *Streptothrix hominis* Foulerton in Foulerton & Jones (**143**, LIII, 79, 87, 100, 1902), pulmonary infection in a woman (case first described but organism not named by Foulerton, **140** (1899 II, 779). As *S. hominis* I, Foulerton **140** (1905 I, 1200; 1906 I, 970), **142**, 309, **140** (1910 I, 553\*, 626-7).

**N. leishmani** Chalmers & Christopherson (**141**, x, 255, 1916), based on *Streptothrix* sp. isolated by Birt & Leishman (**75**, II, 120-8) from a case

- of fatal lung disease and pericarditis in man, **145** (no. 203, 10, 27, 37).
- N. londoniensis** Chalmers & Christopherson (**141**, x, 256, 1916), n.nom. for *Streptothrix hominis* II, Foulerton, **140** (1905 I, 1200), from tongue abscess in man. Foulerton, **140** (1906 I, 970; 1910 I, 553\*, 626-7), **142**, 309.
- N. luteola** (Foulerton) Chalmers & Christopherson (**141**, x, 265, 1916). *Streptothrix luteola* Foulerton, in Foulerton & Jones (**143**, LIII, 75, 86, 93, 1902), from a case of conjunctivitis in man. Foulerton, **140** (1905 I, 1200 (alveolar abscess of the mandible); 1906 I, 970), **142**, 304\*.
- N. macropodidarum** Fox. On Bennett's wallaby (*Macropus bennetti*), Zoological Society of London, Scott **154** (1925, 799-814).
- N. madurae** (Vincent) Blanch. Mycetoma (Madura Foot). On man (Indian): infection contracted in Aden, Duncan *et al.* **155** (xxxii, 427-9, 1939), as *Actinomyces madurae*. **143** (LIII, 87, 96, 1902), as *Streptothrix madurae*; **142**, 303; **140** (1910 I, 551); **146**, 74; **148** (ed. 3, 383-4, 1275).
- N. minutissima** (Burch. & Barends.) Verdun. Erythrasma. (Syn. *Microsporon minutissima*.) On man: Whitfield, **156**, 72\*; **157**, 439-40\*; **158**, 352\*, 363; **159**, 407-8\*.
- N. splenica** Gibson, **160**, 130\*, 1930, from human spleen. Erikson, **145** (no. 203, 36, 1935; cultures 4575, 450, 4579, 4581, 4583), tentatively based five new species on Gibson's cultures of *N. splenica*.
- N. tenuis** Cast. Trichonocardiosis. (Syn. *N. tenuis* Castellani, 1911 (**161**, xxiii, 341); *Discomyces tenuis* [Cast.] Castellani, 1912 (**144**, vi, Derm. Sect. 23-7); *Actinomyces tenuis* (Cast.) C. W. Dodge, 1935 (**152**, 715).) On man (hair). During the summer of 1920-1 over 80% of the patients admitted to the Tropical Section of Orpington Hospital infected, vide Castellani & Wilkinson, **161** (xxxiv, 255-6, 1922; reprinted, **162**, xxvi, 186-90), who distinguished trichomycosis axillaris flava, nigra, and rubra (see also **144**, xvi, 97, and **161**, XLIII, 88; **206**, 140) caused by *Nocardia tenuis* and *N. tenuis* in association with a black pigment-producing coccus (*Micrococcus nigrescens* Cast.) or a red pigment-producing coccus (*M. castellani* Chalm. & O'Farrell), respectively. **162** (xxix, 222); **157**, 443-5\*; **158**, 352.

## DOUBTFUL AND EXCLUDED SPECIES

- Cercospora vexans** Masee in Russ, **140** (1923 I, 77\*), from lesions on palmar surfaces of the forearms of factory workers handling foodstuffs. Listed by Dodge (**152**, 505) as a doubtful synonym of *Ectotrichophyton mentagrophytes* var. *radiolatum* [*Trichophyton mentagrophytes*].
- Lorum uteri** Wilkinson, **140** (1849, 451), from the human uterus. **216**, 367; **182**, 133\*, as *Leptomitius* (?) *muci uteri*; **181**, 65, as *Leptomitius uteri*.



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## INSECTS

[See Petch, List of the entomogenous fungi of Great Britain, 28 (xxxi, 286-304, 1948).]

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A. niger  
Candida tumefaciens

## RAT

Actinomyces muris-ratti

## REPTILES

Aspergillus fumigatus

## SALMON

Saprolegnia ferax  
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NOTES ON *CIRCINELLA SIMPLEX* VAN TIEGHEM

By E. O. CALLEN

*Department of Plant Pathology, Macdonald College, McGill University*

(With 2 Text-figures)

In the spring of 1946 a sample of soil was received in the Department of Mycology, University of Edinburgh. It had been obtained from a castle in Perthshire, Scotland, where a drain in the kitchen regions had become choked, and the soil around it was covered with a slimy mass of fats on which fungi were growing. Cultures were made from this material, and amongst the fungi obtained was *Circinella simplex* van Tieghem.

*C. simplex* appears to have been isolated only twice before—first by van Tieghem (1875) from dog excreta in France, and secondly by Zycha (1935) who obtained it from a soil sample from Brazil. Niethammer (1937) and Gilman (1945) both quote these records without adding to them. Zycha states that the height of the aerial mycelium above the substratum (2–3 mm.) is a constant character which appears to separate it from *C. spinosa*, where it is 20 mm. high. This is really a quotation from van Tieghem's paper (1875, p. 93). In the very next part of the sentence, Zycha adds that this character varies strongly, and that he believes that the two species are identical. The strain isolated from Perthshire has retained its low growth habit through ten subcultures (twenty months) on malt-extract agar, which justifies van Tieghem's separation of these two species.

The figures given by van Tieghem (1875) for *C. simplex* show a single sporangium with a sterile spine, in addition to five sporangiospores and four sporangiophores on a much smaller scale. The complete absence of sterile spines from the fungus from Perthshire made identification somewhat doubtful at first. However, reference to van Tieghem and Le Monnier's paper (1873), in which they first describe the genus, shows that in some of the figures of *C. spinosa* the spines are absent. In their description they do not mention the spines as a special character of the genus, and Zycha (1935) suggests that spine production appears to be greatly influenced by external conditions. It would, therefore, appear that they may or may not be present.

In culture on 2½% malt-extract agar, the aerial mycelium is some 2–3 mm. high and of a fawn colour; buffy citrine according to the Ridgway (1912) colour standards (Pl. 16, colour 19'k). The sporangiophores are of the same colour and are regularly sympodially branched, bearing up to twenty or more fawn-coloured sporangia on short curved branches. According to van Tieghem (1875), the number of sporangia produced is up to fifteen, or even twenty, but in the present material over twenty has been the rule rather than the exception (Fig. 1). On occasions the sporangio-

phores were even branched dichotomously (a condition figured by van Tieghem and Le Monnier (1873) for *C. spinosa*), giving rise to two branches bearing some seven or more sporangia each.

At first a young colony on malt-extract agar resembles a culture of *Mucor*, because of the number of auxiliary sporangia produced, but after three days a small branch can be seen two-thirds of the way up the stronger sporangiophores towards the edge of the culture, and in due course a large sporangium is formed at the end of this branch. Mounting such a young preparation in lactic acid and cotton blue reveals that prior to the formation of the branch, a cross wall is laid down, cutting off the mature first-formed sporangium from the mycelium. On rare occasions, in older



Fig. 1. Sporangiophores and sporangia of *Circinella simplex* v. Tiegh.

cultures (two to three weeks old), some of the auxiliary sporangia may be carried to a height of 5 mm. above the substratum, but they seem to be confined to the immediate area of inoculation.

On mature sporangiophores, the sporangia range in diameter from 3–39  $\mu$  (Zycha 25–50  $\mu$ ), but as the sporangial wall is very brittle, it is difficult to measure the larger sporangia with accuracy. The wall of the sporangium is thickly encrusted with crystals of calcium oxalate.

Sporangia decrease in size as one succeeds another up the sporangiophore. The question naturally arises, if this decrease is regular and gradual, or if it is irregular, and then due to a nutritional factor. Successive sporangia on mature sporangiophores were measured from the base

upwards, and gave the following curves when the decrease in diameter in  $\mu$  of successive sporangia upwards is plotted against unity. In all six series of measurements shown, the readings conclude with the terminal sporangium, and all of them are taken from twelve-day-old Petri dish cultures.

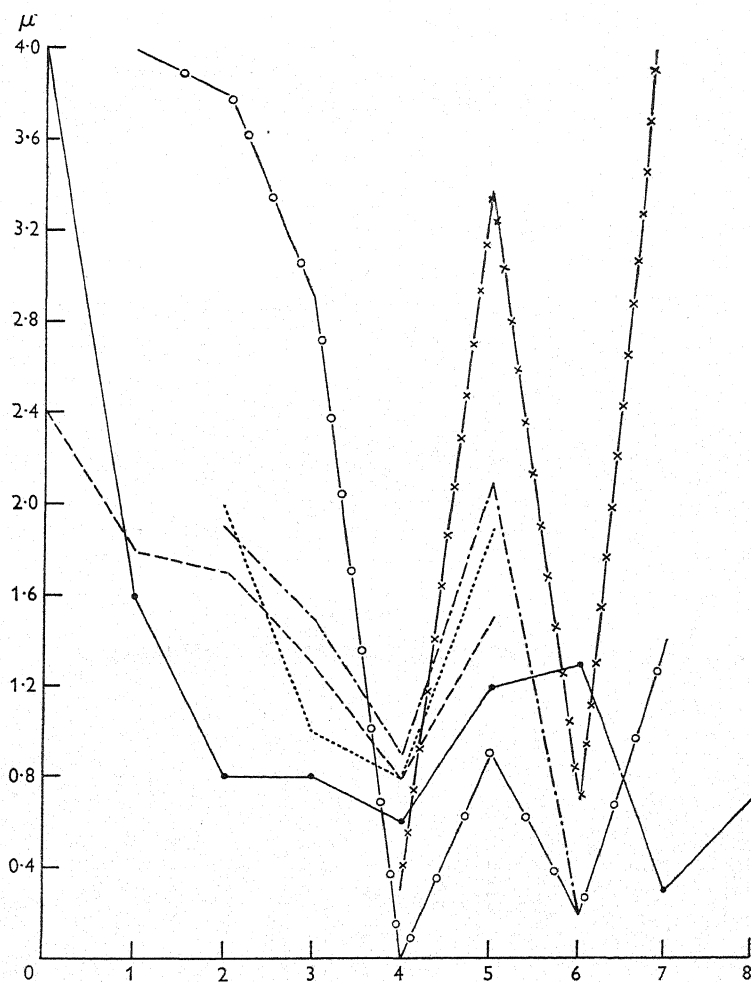


Fig. 2. For description see text.

These six sets of readings show basically the same pattern, the presence of two minima, that is to say, there are two stages during development when successive sporangia on the same sporangiophore are practically identical in diameter. There is a remarkable similarity in the form of these curves, but as can be seen, they do not all begin at the same point. That is to say, the minima do not always occur at the same point on each sporangiophore, though the interval between the two minima is constant. That these



minima do not always occur at the same point is due to the growth of the fungus. Sporangioophores near the edge of the culture are younger than those towards the centre, and as the interval between the two minima is constant, it shows clearly that the main factor governing sporangial size is a nutritional one, with the depth of the medium, and consequent amount of nutriment as the limiting factor.

In a three-day culture on 2½% malt-extract agar, the largest auxiliary sporangium was 19.8 $\mu$  and the smallest 3.2 $\mu$ , with the average of 12.9 $\mu$ . The smallest sporangium contained five spores, though only 3.2 $\mu$  in diameter.

In a sporangium of diameter 25 $\mu$  measured from the tip of its junction with the sporangiophore, the columella was 13 $\mu$  when measured in the same way. In a sporangium of 18 $\mu$ , the columella was 10 $\mu$  measured in the same way.

The spores are hyaline, oval to irregularly oval, 2–4 $\mu$  in size (Zycha 3–5 $\mu$ ), though an occasional giant spore up to 8 $\mu$  has been seen.

This fungus is said to be heterothallic. No zygospores have been seen. Giant cells have not been observed.

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## AQUATIC HYPHOMYCETES FROM SWITZERLAND

By C. T. INGOLD

*Department of Botany, Birkbeck College, London*

(With 3 Text-figures)

Working in Britain I found a special flora of aquatic Hyphomycetes growing on submerged decaying leaves of broad-leaved trees and shrubs in streams and other well-aerated water. Some twenty-five species of these fungi were recognized. Six had previously been described, though rather imperfectly, but their special relationship to submerged leaves had not been appreciated.

My early work (Ingold, 1942) was limited to a small stream in Leicestershire, but later, scattered observations (Ingold, 1943) covering England, Wales and Ireland, showed that this flora was widespread, and suitable decaying leaves from any stream never failed to give crops of these fungi.

The examination of leaves from other localities in the British Isles added only a few to the list of species reported from the stream in Leicestershire, and, although intensive search would almost certainly add a few to the list of species already known, it is doubtful if many more await discovery.

It was clearly of interest to know if the same flora was to be found elsewhere in Europe and, on a short visit to Switzerland in the late summer of 1947, I took the opportunity to study the fungi of submerged decaying leaves of willow and alder in alpine waters. My observations were made possible by the kindness of Prof. E. Gäumann of Zürich who not only lent me a microscope and other necessary apparatus, but also suggested an excellent locality (Surlej-Silvaplane) for my operations.

Surlej (1800 m.) lies in that valley of the Upper Engadine which includes a series of lakes (St Moritzersee, See von Campfer, Silvaplanersee and Silbersee) linked by short stretches of river. Bordering these stretches, on either side of the main channel, are patches of willow scrub, flooded doubtless when the river is in spate, but through which in summer there trickle occasional small streams. Willow leaves from these small streams were examined in the region between St Moritzbad and the See von Campfer. In addition to the main river, there are numerous little torrents cascading down the mountain sides to join the water system of the valley. The steep sides of the valley up to about 2100 m. bear a fine coniferous forest, but bordering the torrents are alders and willows. Decaying leaves of these, caught between the small boulders of the streams, were also examined.

It was at once apparent that the decaying leaves, both from the main stream and from the tributary torrents, bore rich crops of aquatic Hyphomycetes.

Altogether six collections, each of 10-20 leaves, were carefully examined. These yielded nine known species (Table 1 and fig. 1) and, in addition,

an interesting new fungus apparently referable to the genus *Anguillospora*. All these species figured in more than one collection.

Table 1

Species	Notes
<i>Alatospora acuminata</i> Ingold	Very abundant
<i>Lemonniera aquatica</i> de Wild.	Very abundant
<i>Flagellospora curvula</i> Ingold	Very abundant
<i>Tetracladium marchalianum</i> de Wild.	Very abundant
<i>Articulospora tetracladia</i> Ingold	Abundant
<i>Tricladium splendens</i> Ingold	Seen three times
<i>Heliscus aquaticus</i> Ingold	Seen twice
<i>Tetracladium setigerum</i> (Grove) Ingold	Seen twice
<i>Tricladium angulatum</i> Ingold	Seen twice

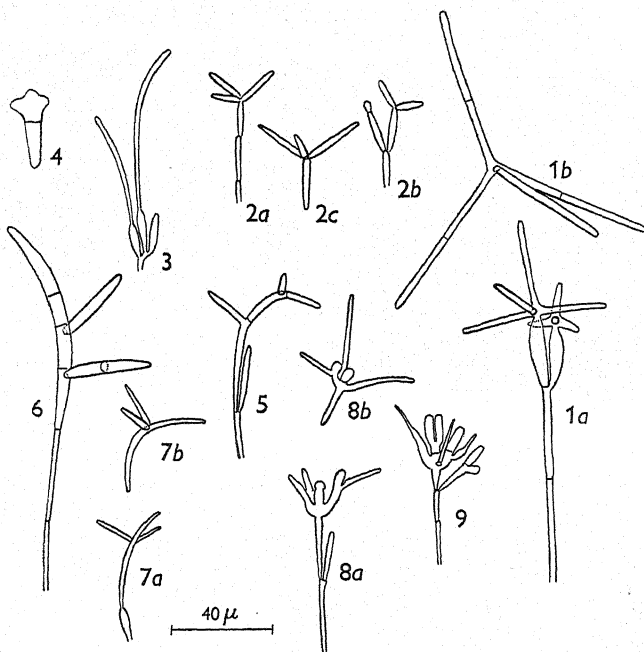


Fig. 1. Aquatic Hyphomycetes drawn with the aid of a camera lucida from specimens collected in Upper Engadine. 1, *Lemonniera aquatica*, a, conidiophore with two developing spores; b, spore. 2, *Articulospora tetracladia*, a, conidiophore with one ripe spore; b, conidiophore with two developing spores; c, spore. 3, *Flagellospora curvula*, conidiophore bearing two spores. 4, *Heliscus aquaticus*, spore. 5, *Tricladium angulatum*, conidiophore with one ripe spore and an unbranched spore-primordium. 6, *Tricladium splendens*, conidiophore with ripe spore. 7, *Alatospora acuminata*, a, conidiophore with ripe spore; b, liberated spore. 8, *Tetracladium marchalianum*, a, conidiophore bearing one nearly mature spore and an unbranched spore-primordium; b, spore. 9, *Tetracladium setigerum*, conidiophore bearing one mature spore and a spore-primordium developing its first lateral branch.

These organisms clearly formed the characteristic fungus flora of the decaying leaves and, although a few pythiaceous fungi were seen, they were few and far between. Submerged decaying leaves of herbaceous plants and of conifers were examined, but no trace of aquatic Hyphomycetes was found on them.

With the exception of *Tetracladium marchalianum* these fungi are recorded for the first time from Switzerland. De Wildeman (1894) reported *T. marchalianum* from a pond in Geneva. However, even the restricted conception of that species which he proposed in 1894 included at least two species (see Ingold, 1942) and, as he gave no figures of the Geneva material, it is not possible to say what species he actually observed.

A noteworthy feature of the list (Table 1) is the absence of *Clavariopsis aquatica* and *Anguillospora longissima* which are almost invariably present on submerged decaying leaves from streams in Britain.

#### *Anguillospora flagellifera* n.sp.

This fungus (Figs. 2 and 3) was found in the first instance on a submerged leaf decayed too far for certain identification, but it was later collected on a decaying willow leaf from another stream.

The mycelium is branched, septate, hyaline and ramifies in the tissue of the leaf, from which the conidiophores grow out at right angles. The conidiophore is simple, narrow ( $2\mu$  diam.) towards its base, but inflated near its apex ( $6-9\mu$  diam.). The single elongated, hyaline conidium (aleuriospore) is terminal. It is  $140-200\mu$  long, truncate at its base where the diameter is  $6-8\mu$ , but it widens upwards becoming  $10-14\mu$  broad at a distance of  $40-50\mu$  from the base, but above that it narrows, to form a whip-like terminal part only  $2-3\mu$  in diameter. This whip-like end is curved in such a way that the spore does not lie in a single plane. The conidium has 4-6 septa and is very pearly in appearance no doubt due to a reserve of glycogen. When mature the spore separates from its conidiophore apparently by the breakdown of the middle lamella at the junction between the two. Germination is by a single, very straight germ tube developed laterally from near the base of the lowermost cell of the spore, quite often before it is shed. The liberation of the spore does not necessarily bring the activity of the conidiophore to an end, for it may grow out laterally, below the scar left by the separation of the first spore, and give rise to a second.

The reference of this well-marked species to the genus *Anguillospora* seems justified because it is an aquatic Hyphomycete with a worm-like spore of the aleuriospore type borne singly on a conidiophore which is usually simple. It differs from the only other species, *A. longissima*, in a number of ways. First, the spore is wider and its two ends are strikingly different, whilst in *A. longissima* it is almost impossible to distinguish base from apex in the liberated conidium. Secondly, the conidium germinates by a single lateral germ-tube arising from the basal cell, but that of *A. longissima* germinates by basal and apical tubes in line with the longitudinal axis of the spore. Thirdly, the conidiophore with its inflated apex contrasts with that of *A. longissima*, for in that species it is difficult to be sure, until the spore is about to be shed, where the conidiophore ends and the conidium begins. Fourthly, no separating cell, such as occurs in *A. longissima*, is developed.

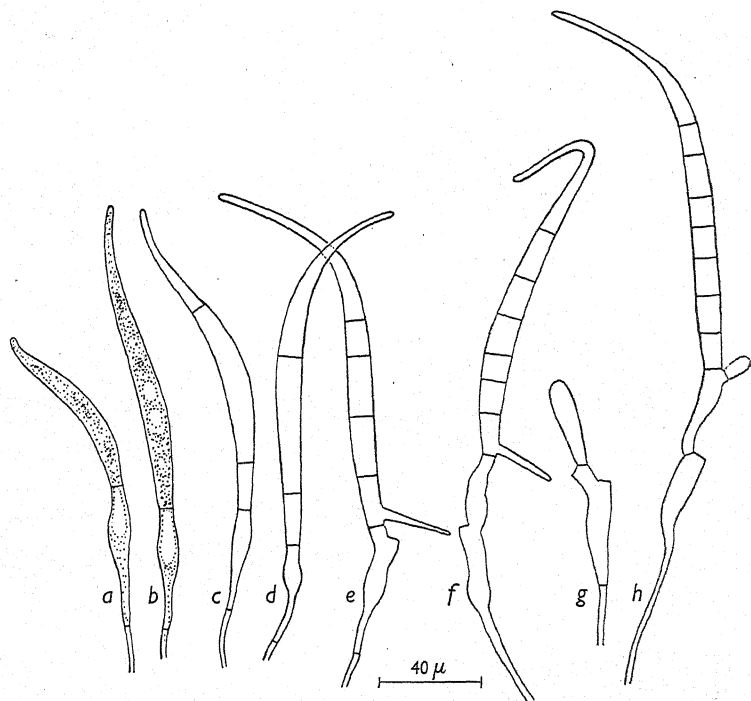


Fig. 2. *Anguillospora flagellifera*. Conidiophores with conidia. Contents shown only in *a* and *b*. In *e* and *f* the conidium has germinated while still attached. In *e*, *f*, *g* and *h* scars left by detached conidia are shown. In *h* a very young spore-primordium is shown to the right of a mature spore.

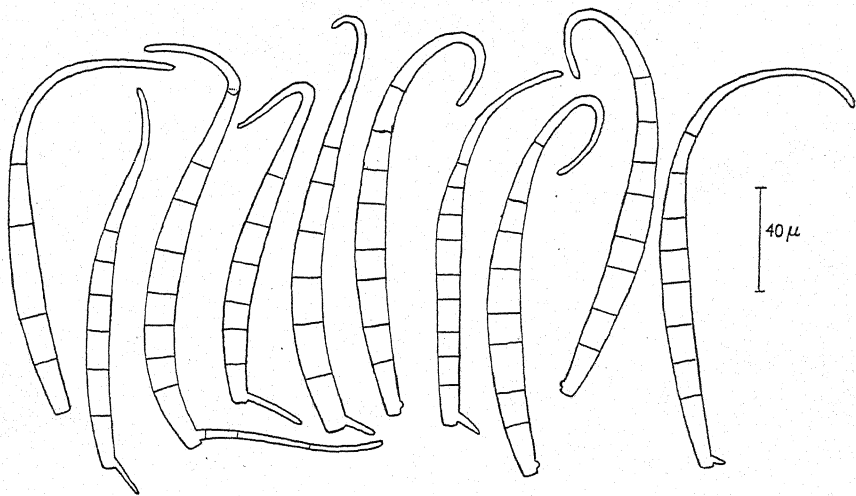


Fig. 3. *Anguillospora flagellifera*. Conidia, most of which have started to germinate.

*Anguillospora flagellifera* sp.nov.

Fungus submersus aquaticus, mycelio repente, hyalino, septato, ramoso. Conidiophora simplex, basim versus  $2\mu$  lata, summa parte inflata  $6-9\mu$  lata. Conidium (aleuriospora) acrogenum, hyalinum,  $4-6$  septatum, vermiforme vel falcatum,  $140-200\mu$  longum, ad basim truncatum  $6-8\mu$  latum, in medio  $10-14\mu$  latum, ad apicem filiforme curvatum et ad  $2-3\mu$  diam. attenuatum. In germinatione oritur una lateralis hypha solum e conidii infima cellula.

*Hab.* In foliis putrescentibus in flumine submersis, in Valle Engadin Helvetiae.

## SUMMARY

Aquatic Hyphomycetes form the characteristic fungus flora of submerged decaying leaves of alder and willow in Switzerland as in Britain. Leaves collected from streams in the Upper Engadine in August 1947 yielded nine species already reported from Britain and, in addition, a species, *Anguillospora flagellifera*, apparently new to science.

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NOTE. Since this paper was accepted, I have found *Anguillospora flagellifera* on a decaying leaf from a small lake at Clandeboyne, Co. Down, Ireland, during the Autumn Foray, 1948.

## REVIEW

*The Fungi.* By F. A. WOLF and F. T. WOLF. New York: John Wiley and Sons; London: Chapman and Hall. 1947. Vol. 1, x+438 pp., 153 t.f., 36s. net; Vol. II, xii+538 pp., 82 t.f., 39s. net.

The first volume of *The Fungi* describes the morphology, development and classification of the fungi, the second treats of the biology and physiology of the living fungus, as well as of such general matters as geographical distribution and the fossil record. In intention, the work is a comprehensive account of what is known about fungi. It should be useful to the working mycologist, for there is little of mycological interest which is not presented, either directly, or by reference to other works. But the reader will need to verify many of the statements which appear in the two volumes.

The authors make it clear that they are mainly interested in the living fungus, and that they consider theoretical developments to be of secondary importance. Perhaps the most striking feature of the work is the introduction of free comments on what may be called botanical politics. It may well be that some readers will regard these comments as immature, or indiscreet, or even as unnecessary. But they have their value, and one chapter (Vol. II, chapter 18), which consists largely of comments, merits special mention. The chapter contains little which has not been said often in private conversation or in public discussion, and little or nothing which will be new to the thoughtful reader, yet it is of value as a careful exposition of matters which are too commonly disregarded.

Of the two volumes, the second appears likely to be the more useful. It contains a stimulating account of the fungus in action. Such an account, in so convenient a compass, does not seem to have been attempted since 1890, when Zopf's *Die Pilze* appeared (in Schenk's *Handbuch der Botanik*, Vol. IV, pp. 271-755). Now, when mycology is developing so rapidly and when the biology and physiology of the fungi come more and more into prominence, this second volume will soon be in need of revision. The authors will benefit their readers by producing a second edition in which additional information will be included, and in which the misprints and mis-statements to be found in the existing volume will have been corrected. It is disappointing to have to grumble at so forward-looking a work as *The Fungi*, but there are regrettable misprints. For instance (Vol. II, p. 87), *Penicillium citrinum* gives citrinin, not penicillin, and flavicin is active against Gram-positive bacteria. The chemical formulae and equations are not always correctly printed, and it is surprising to find *Amanita muscaria* figured as *A. phalloides* (Vol. II, fig. 58) and to find *A. caesarea* included as a common poisonous fungus. Those two troublesome words, 'substratum' and 'synonymy' seem to have defeated the proof readers, and the indexes are not wholly reliable.

Even in these days, £3. 15s. od. for the two volumes is a heavy price. Many students in this country will not be able to afford to buy the books. Maybe, if a revised edition appears, it will not only be more accurate in its facts, but also priced at a level nearer to that possible to the student's purchasing power.

B. BARNES

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